

SCIENCE

SEPTEMBER 8, 1950



COVERAGE OF SCIENTIFIC PERSONNEL
IN AMERICAN MEN OF SCIENCE

M. H. TRYTTEN

THE BIOLOGICAL SYNTHESIS OF
"LABILE METHYL GROUPS"

V. DU VIGNEAUD, C. RESSLER, J. R. RACHELE

TECHNICAL PAPERS

COMMENTS AND COMMUNICATIONS

BOOK REVIEWS

NEWS AND NOTES



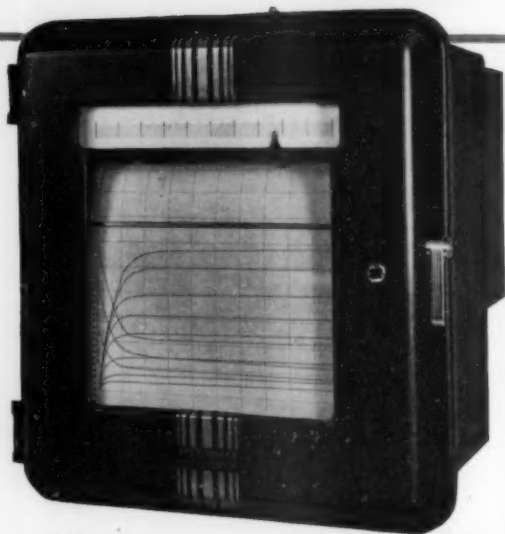
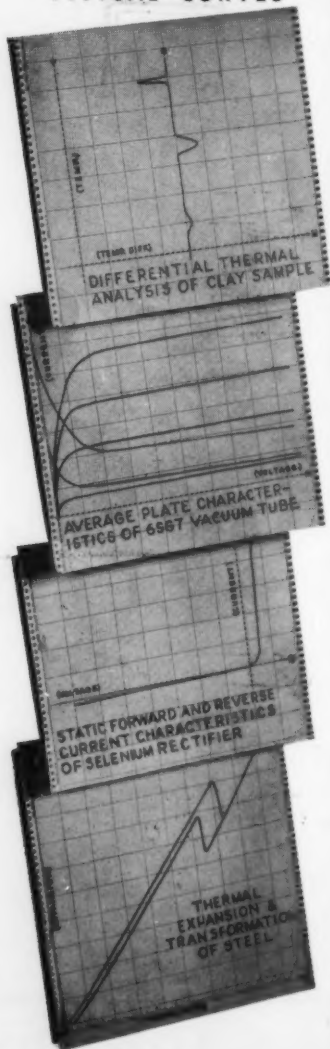
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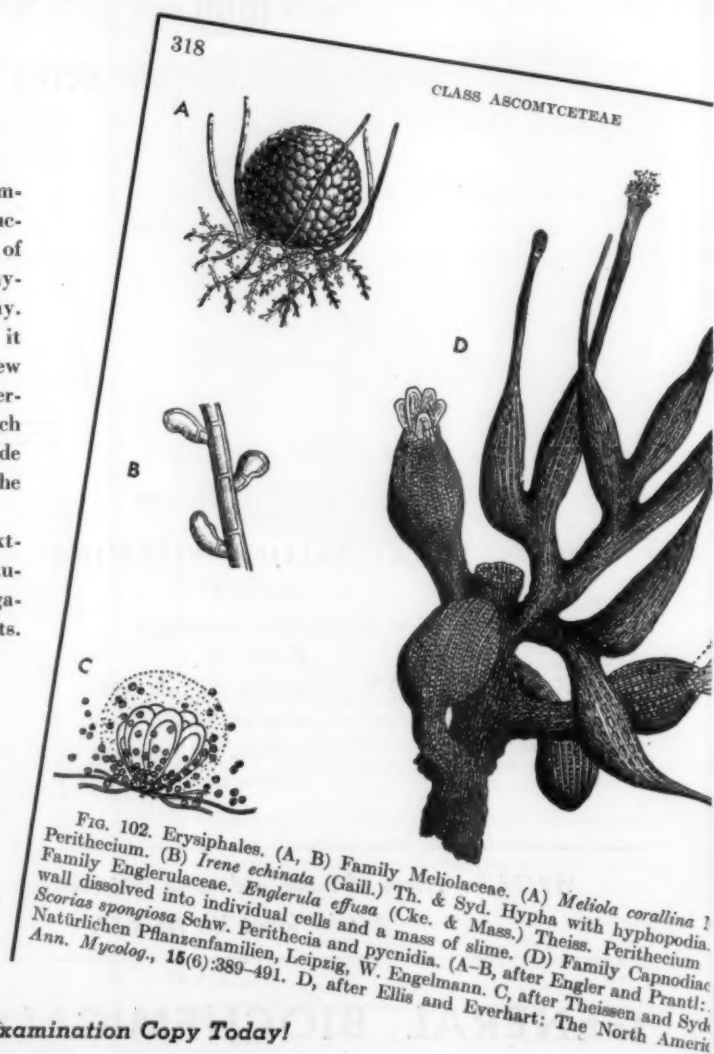
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Table of Contents

Coverage of Scientific Personnel in
American Men of Science, Eighth
Edition: *M. H. Trygten* 265

The Biological Synthesis of "Labile
Methyl Groups": *Vincent du Vigneaud*,
Charlotte Ressler, and *Julian R. Rachele* 267

Technical Papers

Selective Inhibition of Brain
Respirations by Benadryl:
Edith M. Carlisle and *Frederick Crescitelli* 272

Ammoniated Dentifrices and Hamster Caries:
The Effect of Ingestion:
D. S. Chernausk and *David F. Mitchell* 273

Chloromycetin in the Treatment of
"Red Leg": *Stuart W. Smith* 274

A Diploid Form of *Medicago sativa* L.:
J. L. Bolton and *J. E. R. Greenshields* 275

Quantitative Aspects of the Action of
Insulin on the Glucose and Potassium
Metabolism of the Isolated Rat
Diaphragm: *A. F. Willebrands et al.* 277

A Method for Silver Staining of Nerve
Fibers in Whole-Mount Preparations of
Blood Vessels: *Edward H. Polley* 278

Molluscan Shells as a Practical Source of
Uroporphyrin I: *Alex Comfort* 279

Recent Patterns of Employment and
Unemployment: *Nathan Morrison* 280

Comments and Communications

On the Persistence of 2,4-D in
Plant Tissue: *H. B. Tukey* 282

Concept of Complementarities:
Discussions by *Jerome Alexander*,
A. M. Schechtman, and *Toshiko Nishihara* 283

Our Flat Planet: *W. A. Rockie* 283

Book Reviews

Antibiotics: *H. W. Florey, et al.*
Reviewed by *Malcolm H. Soule* 284

Research in Medical Science: *David E.*
Green and *W. Eugene Knox, Eds.* 284

The Nature of Natural History:
Marston Bates 284

News and Notes

Atmospheric Turbulence Discussion:
F. N. Frenkiel 285

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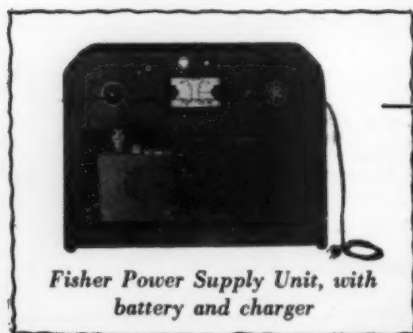
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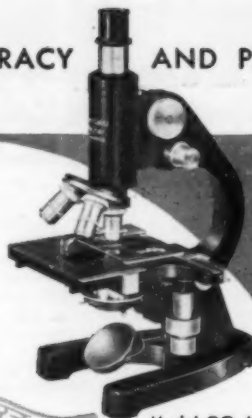
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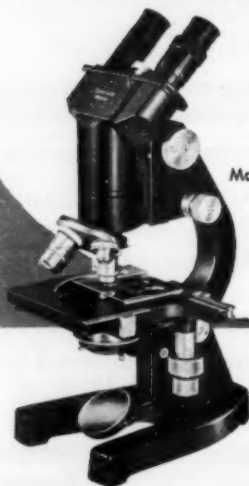
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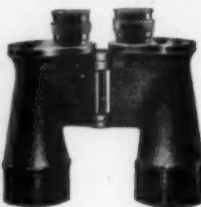
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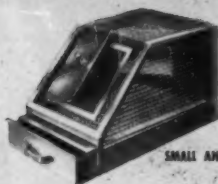
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Coverage of Scientific Personnel in *American Men of Science*, Eighth Edition

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WITH THE TERMINATION of the activities of the National Roster of Scientific and Specialized Personnel following World War II, no active register of scientific personnel was available. Partly to fill the gap, the Office of Naval Research entered into a contract with the National Research Council to secure information on scientists with more advanced training, through cooperation with the publishers of *American Men of Science*. As a result of this effort, registration in *American Men of Science* was sharply increased beyond the number registered in the seventh edition. Approximately 52,600 scientists were registered, and information about them is available. The National Security Resources Board has now taken steps to circularize additional scientists through cooperation with scientific societies, and it is anticipated that this effort will get under way in the next few weeks. The new registration will be integrated with the existing files of persons registered in connection with the publication of *American Men of Science*.

The extent to which the registration in *American Men of Science* covers the scientific population has been of much interest. In general, the editors of *American Men of Science* accept for registration those persons whose training or experience is the equivalent of the attainment of a doctorate in one of the scientific fields.

To determine the extent of coverage, the Office of Naval Research requested the Office of Scientific Personnel, of the National Academy of Sciences-National Research Council, to make a study of various appropriate groups to determine in each case the percentage of coverage. The membership rolls of the American Mathematical Society (November, 1948), American Physical Society (October, 1948), Geological Society of America (December, 1948), American Psychological Association (August, 1949), Botanical Society of America (March, 1950), and Genetics Society of

America (March, 1950) were obtained and studied. These cover various segments of the scientific population only, but it was felt that they would furnish a reasonably satisfactory basis for estimation of coverage in other fields. In those cases where membership might include numbers of people whose level of training or experience was below the level used by the editors of *American Men of Science*, selected lists were developed with the aid of officers of the societies. In the case of the American Psychological Association, which includes branches of psychology where registration in *American Men of Science* is not general, certain specialties were selected. Of these various lists an adequate sample was selected, and the names checked against registration in *American Men of Science*.

J. R. Kline, secretary of the American Mathematical Society, very kindly checked its membership list to show those who might be expected to be included in *American Men of Science*. Approximately each fifth name was checked. In the case of the Geological Society of America, members of fellowship grade were checked for inclusion. Every tenth name was checked. In the case of the American Physical Society, out of a total membership of 7,238, the list of 992 Fellows was used as a base. About one in eight was checked. In the case of the American Psychological Association, the Fellows in the following fields were included: general psychology, teaching, theoretical-experimental, evaluation and measurement, physiological and comparative, childhood and adolescent, clinical and abnormal, military, and maturity and old-age psychology. Approximately each tenth name was checked. In the case of the Botanical Society of America, out of the total of 1,279 members, 200 were selected who were clearly indicated as professional botanists. The total membership list of 1,198 of the Geological Society of America was used, and a 10 percent sample checked. Table 1 indicates

TABLE 1
A. M. S. COVERAGE

Society	No. names	Names checked	Listed in A. M. S.	Total percentage found
American Mathematical Society, doctorates only	1,770	356	270	76
American Physical Society	7,238	902	905	91
American Psychological Association, Fellows in natural sciences	1,234	119	103	87
Botanical Society of America	1,279	200	166	83
Genetics Society of America	647	647	538	83
Geological Society of America	1,198	118	99	84

the membership on the lists selected, number of names that were checked in each list, and the number of those listed in *American Men of Science*; resulting percentages are also shown.

The Office of Scientific Personnel has on file the names and a certain amount of personnel data about all the individuals who have received the doctorate in the natural sciences from 1936 to date. Individuals receiving their doctorate in the fields of biochemistry, chemistry, geology, metallurgy, paleontology, physics, and psychology, for the period 1936-45, inclusive, have been listed separately. Each of these subject matter lists was sampled, giving the results listed in Table 2.

In addition, the file of those receiving the doctorate in 1936-45 in all subject matter fields—14,240 in all—was checked, using every twentieth name. This showed that 75 percent of the individuals receiving the doctorate in the natural sciences during the ten-year period were listed in *American Men of Science*. A similar check made of the over-all group re-

TABLE 2
A. M. S. COVERAGE

	No. names	Names checked	Listed in A. M. S.	Total percentage found
Biochemistry	870	100	76	76
Chemistry	4,905	200	153	76
Geology	398	100	73	73
Metallurgy	118	59	38	64
Paleontology	68	34	28	82
Physics	1,220	124	106	85
Psychology	920	100	58	58
1935-45	14,240	726	553	76
1946-47	1,656	207	169	81
1948-49	4,381	346	117	30

ceiving the doctorate in the natural sciences in 1946-47, inclusive, yielded 81 percent. Those receiving the doctorate in 1948 and 1949, inclusive, however, yielded a coverage of only 30 percent, owing largely to the fact that not all the 1948, and practically none of the 1949, doctorates had an opportunity to be included in *American Men of Science*.

It will be noted from a comparison of Tables 1 and 2 that registration of younger scientists is somewhat less than that of the membership in the comparable groups of members in societies. The coverage in both cases is substantial and represents the preponderance of personnel trained to this level. Some of the failure to achieve a higher percentage of registration resulted from lack of addresses for some persons whose names were known. This was particularly true of more recent holders of a doctorate in which the files of the Office of Scientific Personnel were virtually complete, but for which the most recent addresses were not available. If the present emergency results in a need for a registry of information, the present file will obviously furnish a very important resource of information in dealing with persons of advanced training in the various sciences.



The Biological Synthesis of "Labile Methyl Groups"^{1,2}

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IN THE FIRST REPORT OF GROWTH of the rat on a methionine-free, homocystine-containing diet supplemented with choline, in which the concept of the "biologically labile" methyl group and transmethylation was proposed, du Vigneaud, Chandler, Moyer, and Keppel (8) called attention to the fact that they occasionally encountered animals "capable of showing some growth on the homocystine diet without added choline." These authors stated: "It appears to us that the explanation of this behavior probably lies in the phenomenon of refection, or else certain factors are operating of which we are not as yet cognizant." It was well known that refection caused difficulties in the assay of the various members of the vitamin B complex. The tendency was, therefore, to attribute growth of some animals on a methyl-free diet to the synthesis of biologically labile methyl groups by intestinal bacteria.

Several years later, Toennies, Bennett, and Medes (30, 3) reported that they had repeatedly encountered growth of rats receiving methyl-free, homocystine-containing diets. When they administered Sulfasuxidine along with eight B vitamins, however, growth of the animals was interrupted (1). Addition of a "folie acid" concentrate and extra biotin did not cause a re-

sumption of growth. However, when a crude liver extract was added to the methyl-free diet in place of the B vitamins, growth ensued that could not be accounted for by the methionine and choline contents of the extract. As pointed out by Bennett, Medes, and Toennies (3), "There may be vitamin factors, of either dietary or intestinal origin, the presence of which may enable the animal to compensate for the absence of dietary methyl donors by biosynthetic means of its own or of its intestinal bacteria."

The problem was further investigated in this laboratory in an attempt to get crucial proof of the synthesis of biologically labile methyl groups in the animal, regardless of whether it took place in the tissues or in the intestinal tract. This was accomplished in an experiment with rats, in which the deuterium concentration of the body water was kept at a level of about 3 atom percent for a period of several weeks (11). At the end of this time the animals were sacrificed, and the choline was isolated from the tissues and degraded to trimethylamine. It was felt that, if, under these conditions, deuterium were found in the methyl groups of choline, synthesis of the methyl group must have occurred in the animal tissues or in the intestinal tract. It was extremely unlikely that a direct exchange reaction between the hydrogens of biologically labile methyl groups and the deuterium of the D₂O in the tissue fluids could take place to bring about the appearance of the deuterium in the methyl group. Strong evidence of this unlikelihood had already been presented (9) and has now been further strengthened by more recent studies with methionine, in which the methyl group was doubly labeled with C¹⁴ and deuterium (19). The concentration of deuterium in the methyl groups of choline isolated from the two rats kept on heavy water rose to 7.7 and 8.5 percent of the concentration of deuterium in the body water. It was pointed out that

The present data do not distinguish between direct synthesis by the tissues and synthesis by intestinal bacteria with subsequent utilization of the methyl groups in the tissues. On the basis of the facts we now have con-

¹ The crucial experiment reported herein on germ-free animals was made possible through the collaboration of James A. Reyniers, Thomas D. Luckey, and their co-workers at LOBUND, of the University of Notre Dame. We are extremely indebted to them. At the suggestion of Professor Reyniers, this paper is submitted by us as a résumé of the present status of the synthesis of the biologically labile methyl groups, in which the results on choline from this joint experiment are incorporated. It is planned to submit jointly elsewhere the complete bacteriological and chemical details of the experiments, involving not only the choline data but also further work on the feces, urines, and carcasses.

² While these experiments were under way, it was announced by H. G. Wood in a Harvey Lecture, February 16, 1950, that W. Sakami and A. D. Welch have demonstrated the synthesis of biologically labile methyl groups from formate *in vivo* in the rat and by rat liver slices *in vitro*. Our results with the germ-free animals are thus in harmony with their result demonstrating tissue synthesis of biologically labile methyl groups. Since the submission of this article, their abstracts recording this work have appeared (Sakami, W., *Fed. Proc.*, 1950, 9, 222; Welch, A. D., and Sakami, W., *Fed. Proc.*, 1950, 9, 245).

cerning the labile methyl groups in the diet and in metabolism, we feel that the latter explanation involving intestinal bacteria is the most logical interpretation of our results.

In an attempt to explain the greater frequency of growth on the methyl-free, homocystine-containing diet encountered at the Lankenau Laboratories (30, 3), the Cornell group furnished the Lankenau group with animals from their stock. Litters from these animals were maintained at the Lankenau Laboratories on their experimental regimen (4). The litters behaved similarly to the Lankenau strain, thus ruling out strain differences and indicating the importance of preexperimental nutritional conditions for the capacity to grow under these conditions. Bennett and Toennies (4) were also able to show that modification of intestinal flora by sulfonamide action produced a situation in which supplementation of the dietary vitamins with rice polishings extract permitted the utilization of homocystine in lieu of methionine when either choline or small amounts of certain liver fractions were fed. Further work explored the nature of the liver factor. A comparison of a variety of liver preparations led to the conclusion by Bennett and Toennies that it was not identical with the antipernicious anemia principle of liver, although its distribution paralleled that of the latter to some degree.

While these studies on homocystine and methionine were under way, other evidence that might have a bearing on the problem emerged. It began to appear that there is a relation between vitamin B₁₂ and the metabolism of biologically labile methyl compounds. That the animal protein factor influenced the methionine requirement of chicks was shown by Patton and co-workers (22) and by Bird and co-workers (5). Furthermore, Shive (28) found an interrelationship between vitamin B₁₂ and methionine in bacterial metabolism. He reported that these two compounds could function interchangeably in enabling growth of *Escherichia coli* to take place in a medium containing sulfanilamide. The sparing action of vitamin B₁₂ on the dietary choline necessary for increased growth and protection against the hemorrhagic kidney syndrome in the rat was noted by Schaefer, Salmon, and Strength (26). Gillis and Norris (15) reported that the inclusion of a source of animal protein factor in their basal diet obviated the need for supplementary methylating compounds for chicks and stated that at least one metabolic function of the animal protein factor was concerned with transmethylation. Bennett (2) then reported that vitamin B₁₂ plus folic acid gave an effect similar to that obtained earlier with the crude liver extracts with rats on the methyl-free, homocystine type of diet. Stekol and Weiss (29) also reported that young rats were able to grow on a labile

methyl-free diet that contained vitamin B₁₂ and homocystine. At the same time Jukes and Stokstad (17) reported that vitamin B₁₂ was involved in the choline and methionine requirements of chicks.

At this stage of the investigation it was clear that labile methyl groups could be synthesized somewhere in the animal body, as evidenced by the observation of growth on a methyl-free diet under certain conditions, and by the demonstration of the formation of deuteriomethyl groups by animals in which the body fluids contained D₂O. It also appeared highly probable that vitamin B₁₂ and folic acid might be involved in the synthesis of these labile methyl groups. But whether the methyl groups were synthesized in the tissues of the white rat, or whether vitamin factors mediated through the intestinal bacteria, was not clear. In every experiment reported, these two interpretations were possible. The present investigation with germ-free animals was arranged in order to obtain crucial evidence as to where the synthesis took place. The technique of detection of synthesis of methyl groups through the isolation of choline from rats whose body water contained D₂O was again utilized.

The plan of the experiment was to maintain rats of the LOBUND strain with D₂O in their drinking water under both germ-free and nonsterile conditions at LOBUND of the University of Notre Dame, and to maintain at the Cornell laboratory, for comparative purposes, animals of the Rockland Farms strain under the usual laboratory conditions on the same dietary regimen as that used at LOBUND.

Two male, germ-free rats reared by the technique of Reyniers were maintained in individual metabolic compartments under germ-free conditions at LOBUND (23). The procedure permitted the administration of food and water, and the collection of the urine and feces under sterile conditions. Sterilized drinking water containing 10 atom percent D₂O was furnished for 4 days, followed by 4 atom percent D₂O for the rest of the experimental period. An average deuterium level in the body water of approximately 2.5 atom percent during the experiment resulted. The diet employed was essentially that already elaborated at LOBUND for the rearing of animals under germ-free conditions.² At the end of the periods given in Table 1 the germ-free animals were sacrificed under sterile conditions. Samples of

² The diet had the following composition: casein (Labco) 25 g, cellophane 2 g, corn starch 59.5 g, salt mixture 6 g, corn oil 7 g; vitamins/100 g of diet: vitamin A (esters) 800 I.U., vitamin D₃ 100 I.U., vitamin C 200 mg, vitamin E 50 mg, vitamin K 10 mg, inositol 100 mg, thiamin hydrochloride 6 mg, riboflavin 3 mg, pyridoxin hydrochloride 2 mg, pyridoxamine hydrochloride 0.4 mg, calcium pantothenate 30 mg, nicotinamide 5 mg, nicotinic acid 5 mg, biotin 0.1 mg, folic acid 1 mg, p-aminobenzoic acid 5 mg, vitamin B₁₂ 0.01 mg.

TABLE 1
DEUTERIUM CONTENT OF METHYL GROUPS OF CHOLINE

Rat No.	Experimental period	Change in body weight	Choline chloroplatinate		Trimethylamine chloroplatinate (A)		Deuterium in methyl groups of choline (B)*	Deuterium in body water (average) (C)	$\frac{B}{C} \times 100$	
	days	gm	mg	% Pt†	% Pt‡	atom % excess D‡	atom % excess D	atom % excess D§		
CUMC‡, ¶	710	21	119-237	469	32.1	37.1	0.23	0.26	3.29	7.8
CUMC‡	707	21	109-186	429	31.7	36.7	0.19	0.21	3.08	6.9
CUMC	708	21	103-186	419	32.2	36.7	0.19	0.21	2.52	8.4
CUMC	709	21	105-225	462	31.2	36.8	0.21	0.23	3.01	7.8
LOBUND	1	21	183-253	533	31.4	36.9	0.19	0.21	2.20	9.6
LOBUND	2	21	180-282	579	31.2	37.0	0.18	0.20	2.27	8.8
LOBUND (germ-free)	5	23	294-322	661	31.4	36.8	-0.14	0.16	2.44	6.4
LOBUND (germ-free)	6	10	308-289	632	31.4	37.2	0.09	0.10	2.99	3.3

* Calculated value: $B = \frac{10}{9} A$.

† Theoretical Pt content for choline chloroplatinate = 31.7%; for trimethylamine chloroplatinate = 37.0%.

‡ Determined with mass spectrometer (% error = ± 0.02).

§ Determined by falling-drop procedure (% error = ± 0.06).

¶ CUMC = Cornell University Medical College, Rockland Farms strain.

§ Received additional 1.5 μ g of vitamin B₁₂ daily.

the tissues and intestinal contents were examined for the presence of bacteria. No bacterial contamination was detected. The tissues were then ground under sterile conditions, ethyl alcohol was added, and the mixture was shipped along with the sterile urine and feces, by air express to New York.

To obtain the average concentration of heavy water present in the body fluids during the experimental period, the deuterium content of the urine after suitable purification was determined by the falling-drop procedure. The choline was extracted from the tissues of the sterile rats and degraded to trimethylamine with alkaline permanganate (20). The deuterium content of the trimethylamine salt was obtained by a micro deuterium method, which involves the combustion of several milligrams of the salt, reduction of the resulting H₂O-D₂O with zinc, and determination of the ratio of the deuterium-hydrogen mixture by means of the mass spectrometer (24).

The two animals maintained at LOBUND under nonsterile conditions were sacrificed at the times indicated in Table 1, and processed in the same manner as the sterile animals. In order to compare the colony of animals under nonsterile conditions at LOBUND with the colony and experimental conditions at the Cornell Laboratories, two animals were maintained at Cornell on the same diet as that used at LOBUND. The experimental diet was made up at the Notre Dame Laboratories and shipped by air express to New York to eliminate any possible difference in source of dietary material. Another pair of animals was maintained at Cornell on the LOBUND diet, which

already contained generous amounts of vitamin B₁₂ and folic acid, with an additional supplement of B₁₂.

From the data presented in Table 1 it is obvious that methyl groups have been synthesized by the germ-free animals. The enrichment of deuterium in the methyl groups of the choline isolated from the LOBUND germ-free rat after 23 days was 6.4 percent of that in the body water. In the 10-day experiment involving the rat under germ-free conditions, a value of 3.3 percent was obtained, an amount that agrees quite well with that of the longer experiment. A somewhat higher value was obtained in the LOBUND animals under nonsterile conditions, but this may not be a significant difference. On the other hand, it may well be that additional synthesis by intestinal bacteria of labile methyl groups took place in the case of the nonsterile animals. However, the germ-free animals were older. Of ultimate importance, of course, is the fact that deuterium was present in significant amounts in the methyl groups of choline in the germ-free animals. It is also notable that the strain of animals maintained at Cornell on the LOBUND diet synthesized methyl groups to about the same degree as the LOBUND animals at Notre Dame. The additional amount of vitamin B₁₂ administered to two of the Cornell animals did not increase the degree of methyl synthesis.

We believe that the data obtained with the germ-free animals justify the conclusion that biologically labile methyl groups can be synthesized by the tissues of the rat. It might be of interest to review briefly the significance of the establishment of the tissue syn-

thesis of biologically labile methyl groups in relation to the earlier work. In the original paper, in which it was shown that homocystine plus choline could serve in lieu of methionine for growth purposes in the rat, it was noticed that an occasional animal could grow without choline, although the vast majority required a source of labile methyl groups (8). It was inferred that the latter were synthesized by intestinal bacteria, but it is now obvious that they were being synthesized, at least in most part, in the tissues rather than in the intestinal tract by the bacteria. The suggestion first put forth by the Lankenau group that vitamin factors may be involved in the synthesis of labile methyl groups in the tissues of the rat would appear to offer an explanation of these results. It is now realized that the diet employed in this early work was extremely low in vitamin B₁₂ and folic acid. If vitamin B₁₂ and folic acid bear a relationship to labile methyl metabolism in the tissues, the possibility exists that the occasional animal that did grow may have been enabled to do so because of bacterial synthesis of these vitamin factors resulting from a difference in the intestinal flora. On the other hand, a difference in degree of storage of these vitamin factors from the preexperimental period could be involved. The more frequently encountered growth in the Lankenau Laboratories may be attributable, as suggested by the Lankenau workers, to the fact that their preexperimental diet carried relatively large amounts of vitamin factors which may have been stored in the liver and later used in the experimental period. This is consistent with the now well-known storage of vitamin B₁₂. Of course, the preexperimental diet, as these workers well realized, may also have affected the flora of the intestinal tract in such a way as to favor the synthesis of these vitamin factors. Earlier cases of growth on diets low in methyl groups with homocystine in lieu of methionine may be recalled. White and Beach (31), on adding homocystine to an arachin diet low in methionine (0.5 percent) and supplemented with Ryzamin B and powdered liver extract, LEL No. 343, obtained growth almost equivalent to that produced by an equal amount of methionine. Brand (6) also demonstrated growth with homocystine in lieu of methionine on a purified amino acid diet with a vitamin supplement of dried yeast and milk concentrate. It is also interesting that, although Rose and Rice (25), like du Vigneaud, Dyer, and Kies (10), could not obtain growth on a purified diet containing homocystine as the sole source of dietary sulfur with the purified B vitamins and the small amounts of Ryzamin B used, they did report sub-optimal growth on a diet with homocystine and a vitamin supplement of tikitiki and milk concentrate. What part of this growth response was due to the

labile methyl compounds, and what part to vitamin factors present in the diets employed, is difficult to evaluate fully.

Since the present work was undertaken, other reports have strengthened the possibility of a relationship between vitamin B₁₂ and folic acid and the metabolism of the biologically labile methyl group. The additive action of vitamin B₁₂ and folic acid on sparing choline was measured by Schaefer, Salmon, Strength, and Copeland (27) by determining the protection these factors afforded against renal kidney damage in rats and against perosis in chicks on a diet low in labile methyl-containing compounds. In a very recent report Jukes and co-workers (18) have shown that vitamin B₁₂-deficient chicks were unable to utilize homocystine or homocystine plus betaine in lieu of methionine for growth, but could do so with vitamin B₁₂ as a supplement. It has recently been observed by Davis and Mingioli (7) that vitamin B₁₂ is capable of replacing the methionine requirements of an *E. coli* mutant "blocked" in the methylation of homocysteine.

In one of the earlier studies on transmethylation in 1941, methionine labeled with deuterium in the methyl group was administered to rats on an amino acid diet, otherwise free of labile methyl groups, for approximately 3 months (9). This diet, in the light of present knowledge, must have been low in vitamin B₁₂ and folic acid. At the end of the experimental period, the concentration of deuterium in the methyl groups of the choline and the creatine from the tissues rose to 89% of that of the deuterium in the methyl groups of the administered methionine. Obviously considerable transmethylation had occurred during this period. In addition to ruling out formaldehyde as an intermediate in transmethylation, this high concentration of deuteriomethyl groups in choline and creatine would lead one to suspect that not much synthesis of methyl groups had occurred under these conditions.

A consideration of the tissue synthesis of biologically labile methyl groups naturally raises the question as to the nature of the precursors or intermediates in the synthesis. It has been reported by du Vigneaud and Verly (12) that methyl alcohol may serve as a precursor of the labile methyl groups. In an extension of the study of the one-carbon compounds from this standpoint, it has been found that the carbon labeled with C¹⁴ of formaldehyde or formic acid⁴ makes its appearance in the methyl groups of choline (14). However, labeled bicarbonate gave a negative result. The effect of the level of methionine and of other labile methyl compounds on the degree of synthesis is yet to be explored. The level of methionine in the

⁴ In the presentation of their paper on formate, Welch and Sakami also reported formaldehyde as a precursor of labile methyl groups.

diet affects tremendously the rate of oxidation of the labile methyl group to CO_2 (21), and it may well be that a large supply of these methyl groups in the diet brings about some inhibition of synthesis and favors oxidation of potential precursors.

In recent experiments in which the methyl group of methanol was doubly labeled with C^{14} and deuterium (13), the ratio of deuterium to radiocarbon in the methyl group of choline was considerably less than that in the methanol administered. This indicates that oxidation of methanol, followed by reductive conversion to the biologically labile methyl group, occurred probably through formaldehyde and/or formic acid.

The experience at Cornell Laboratories has been that even when fairly high levels of B_{12} and folic acid are present, a source of labile methyl groups must be present in the diet immediately after weaning for the majority of animals to survive. From the work of Griffith (16) it is known that the demand for choline is higher in the young rat shortly after weaning than later, to prevent hemorrhagic kidney.

Undoubtedly, some synthesis of the methyl group at this critical stage does take place but, in the majority of animals, apparently at a rate not fast enough to support normal development of the young animal. These results tend to place the biologically labile methyl groups in the same position that arginine holds with respect to the concept of the essential amino acids, in that it can be synthesized but not at a rate fast enough for the demands of the young growing rat. Thus, in this sense, it can be regarded as an essential dietary component. A supply of preformed labile methyl groups in the diet may also become of considerable significance under special dietary or pathological conditions. Finally, it might be added that the behavior of the biologically labile methyl group that is synthesized within the body and used in the synthesis of a labile methyl compound is not distinguishable, so far as we know, from that of the biologically labile methyl group that is presented to the body in the diet from the standpoint of the metabolic process of transmethylation.

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Technical Papers

Selective Inhibition of Brain Respirations by Benadryl¹

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The following is a preliminary account of some experiments that form part of a larger program to examine the mode of action of antihistamines on the nervous system. It is becoming increasingly clear that the so-called antihistamines have effects in addition to those concerned with blocking the actions of histamine. Nerve cells appear to be especially obvious sites of action for the antihistaminic agents. Numerous investigators have called attention to the drowsiness, the hyperexcitability, the tremors, and the convulsions that may follow the administration of various doses of these compounds (4, 5). The local anesthetic action, as well as the ability of the antihistamines to block nerve impulses in frog nerve fibers, has been reported (1, 2, 6).

The mechanism by which these compounds produce their effects on nerve cells is quite unknown. It is obvious that the metabolic actions of the antihistamines will have to be examined in order to supply a foundation upon which theories of the mechanism of action may be based. Thus far, biochemical studies of the antihistamines are few, although recently the inhibition by pyribenzamine of glucose and pyruvate oxidation and of anaerobic glycolysis in mouse brain homogenates has been reported by Hubbard and Goldbaum (3). The experiments to be reported here will demonstrate an interesting selective inhibitory effect of benadryl hydrochloride on the respiration of slices of rat cerebral cortex in a medium containing *l*-glutamate, in contrast to the respiration in a glucose medium.

The selective effect is summarized in Fig. 1. The oxygen consumption of slices respiring in a Krebs-Ringer phosphate solution (pH 7.35) with glucose and *l*-glutamate as substrates is shown in Curves 2 (glucose) and 3 (glutamate). The oxygen consumption with no added substrate (endogenous) is given in Curve 5. Each of these curves is the curve of best fit (by inspection) through the experimental points. Each experimental point represents the mean of duplicate readings. To avoid confusion the experimental points have not been included. The absence of effect of benadryl hydrochloride (0.0018 molar) on the slices respiring in a glucose medium is shown in Curve 1. It is clear that the

addition of the benadryl at the 60-min point (A) produced no decrease in the rate of oxygen consumption. In contrast, the same concentration of benadryl hydrochloride completely blocked the oxygen consumption of the slices respiring in *l*-glutamate (Curve 4). This differentiation between the respiration in glucose and in *l*-glutamate was obtained in 17 experiments. The selectivity was especially striking when the benadryl hydrochloride was used in the concentration range of 0.0004–0.0018 molar. Concentrations above 0.0018 molar caused some inhibition of the glucose respiration. It is especially noteworthy that the endogenous respiration behaved in response to benadryl hydrochloride, not like respiration in glucose, but like respiration in *l*-glutamate (Curve 6). Results similar to the above were obtained also with pyribenzamine hydrochloride and with histadyl, but a thorough survey of the antihistamines and related substances remains to be done.

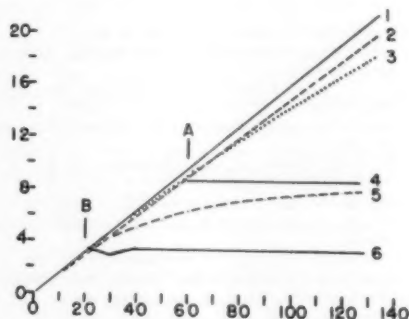


FIG. 1. The action of benadryl hydrochloride on the respiration of rat cerebral slices. Oxygen consumption (μ l/mg dry wt) is plotted against time (min). Concentration of solutions: benadryl hydrochloride, 0.0018 molar; glucose, 0.011 molar; *l*-glutamate, 0.011 molar. Final volume, 0.9 ml; temperature, 35° C; pH, 7.35; gas phase, oxygen. Curve 1, glucose with benadryl hydrochloride added at 60 min (A); 2, glucose control; 3, glutamate control; 4, glutamate with benadryl hydrochloride added at 60 min (A); 5, endogenous respiration; 6, endogenous respiration, benadryl hydrochloride added at 20 min (B).

This differentiation of glucose and *l*-glutamate respiration was not obtained with certain other substances, which also depress the oxygen consumption of cortical slices. For example, *n*-amyl carbamate, *dl*-amidonone, and morphine did not show the selective action of benadryl, the same inhibition being obtained in both glucose and *l*-glutamate media at all effective concentrations of these substances. The outcome of adding *n*-amyl carbamate (0.009 molar) is shown in Fig. 2.

It is of interest to inquire whether the inhibitory action of benadryl hydrochloride on brain slices respiring in *l*-glutamate can be reversed by means of histamine.

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² With the technical assistance of Philip B. Hollander, of the Department of Zoology, University of California, Los Angeles.

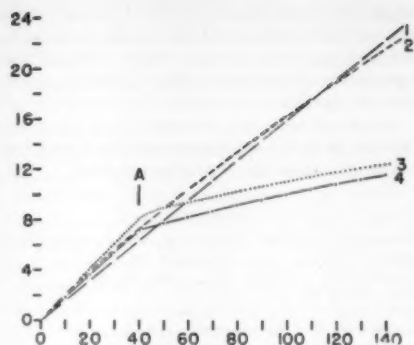


FIG. 2. The action of *n*-amyl carbamate. Solutions same as in FIG. 1, except for *n*-amyl carbamate (0.009 molar). Curve 1, glucose control; 2, glutamate control; 3, glucose with amyl carbamate added at 40 min (A); 4, glutamate with amyl carbamate added at 40 min (A).

That this cannot be done is illustrated in Fig. 3. Histamine dihydrochloride (pH adjusted to 7.35) at a concentration of 0.03 molar did not influence the rate of oxygen consumption of brain slices respiring in glutamate (Curve 2). Neither did histamine dihydrochloride prevent the inhibition of respiration by benadryl hydrochloride (Curve 4).

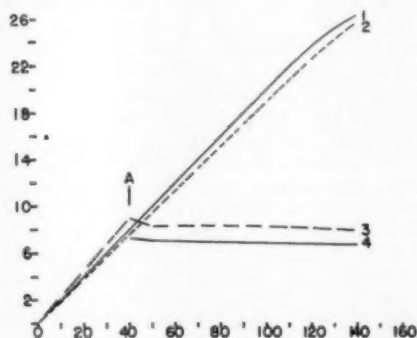


FIG. 3. Failure of histamine dihydrochloride to reverse benadryl effect. Solutions and conditions same as Fig. 1 except for histamine dihydrochloride (0.03 molar). Curve 1, glutamate control; 2, glutamate and histamine dihydrochloride; 3, glutamate with benadryl hydrochloride added at 40 min (A); 4, glutamate and histamine dihydrochloride with benadryl hydrochloride added at 40 min (A).

An analysis of the mechanism by which benadryl produces this selective inhibition of respiration will have to await the outcome of experiments now in progress. Obviously, selective alterations in permeability produced by the benadryl must be considered as a possible mode of action. The inhibition of respiration in experiments with *L*-glutamate and without added substrate makes the permeability concept unlikely, but experiments with brain homogenates, now in progress, should settle this point. If permeability is ruled out, the possibility exists that some metabolic step in the oxidation of *L*-glutamate

is interfered with. Experiments with various metabolites linked to glutamic acid should prove useful in elucidating this point. All these matters will be discussed in a later report after the results of current experiments become available.

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Ammoniated Dentifrices and Hamster Caries: The Effect of Ingestion

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Experimental dental caries has been studied extensively in the Syrian hamster. Arnold (1) and Shafer (6) have shown that this animal is susceptible to dental caries when placed on a high-carbohydrate diet. Keyes (4) believes the carious process in hamsters is fundamentally the same as that observed in human beings.

Recently, ammoniated products have been proposed as an aid in controlling dental caries. Studies *in vitro* seem to indicate that an inhibitory effect on dental caries is possible. There has been very little actual testing of the effect of these products on caries in persons or in animals. Kesel (3) reported encouraging results from the use of a dentifrice and mouth rinse containing ammonia, but stated that further studies are necessary for conclusive results. Stephan (7) has shown that the application of urea in 40%-50% concentrations overcomes the effect of carbohydrate in lowering the pH of the dental plaque material. Keyes (5) studied the effect of weekly topical applications of a 50% urea solution on hamster teeth and found no apparent beneficial results.

It was thought desirable to devise an experiment in which the effect of the ammoniated compounds would be evaluated in regard to caries activity in the hamster. The effect of daily brushing with nonammoniated and ammoniated dentifrices, as well as the effect of ingestion, was studied. This report considers solely the effect of ingestion of dibasic ammonium phosphate and urea on caries incidence in the Syrian hamster.

The hamsters used were from an inbred colony. The animals were weaned at 21 days and placed on a diet of Purina Fox Checkers (with a weekly supplement of fresh carrots and hamburger meat), until they were approximately 30 days of age. The animals were separated into the experimental groups by sex and littermates.

¹The authors are indebted to Jean M. Hartman, Department of Biostatistics, University of Minnesota, for aid in computing the statistics.

They were then placed on a caries-producing diet,² and libitum, for 100 days, after which they were sacrificed and their caries scored and recorded by the method of Keyes (3). The control group (14♀ and 14♂) received the caries-producing diet only, and the ingestion group (13♀ and 12♂) received the same diet plus dibasic ammonium phosphate 1% and urea 0.6%—the ratio commonly found in ammoniated dentifrices.

TABLE 1

Caries scores					
	No. of animals	Mean caries score	Standard deviation	t	p
<i>Female</i>					
control	14	26.4	40.0	2.11*	.05
ingestion	13	1.9	5.2		
Littermates					
control	7	36.8	46.6	1.93†	.10
ingestion					
<i>Male</i>					
control	14	57.5	76.3	2.03*	.05
ingestion	12	10.0	16.4		
Littermates					
control	9	66.1	83.4	2.24†	.06
ingestion					
<i>Male and female groups combined</i>					
control	28	42.0	62.0	2.77*	.006
ingestion	25	5.8	12.0		
Littermates					
control	16	53.3	71.2	2.90†	.011
ingestion					

$$* t = \frac{\bar{x} - \bar{y}}{SE_{\bar{x} - \bar{y}}} \quad \text{where } SE_{\bar{x} - \bar{y}} = \sqrt{\frac{\hat{\sigma}_x^2}{N_x} + \frac{\hat{\sigma}_y^2}{N_y}}$$

$$\text{where } \hat{\sigma}_x^2 = \frac{N_x s_x^2 + N_y s_y^2}{N_x + N_y - 2}$$

$$\dagger t = \frac{\bar{d}}{SE_{\bar{d}}} = \frac{\bar{d}}{\sqrt{N-1}}$$

From the mean caries score in Table 1, it appears that a striking inhibition of caries has occurred. The variation is considerable, however, and, in general, more than reported in other studies. It is possible that, if the animals had continued longer on the diet, the scores would have been higher and possibly more homogeneous. We are assuming a 1% level of significance when testing the difference between two means because of the small number of animals in the groups, and the relative infrequency of valid statistical evaluation of previously published experimental caries data. In addition, one must keep in mind the possibility that the effect of ingestion of these compounds by hamsters may not be comparable to the effect produced in human beings by these compounds.

Previous reports generally have indicated a sex difference in caries activity, if the animals are maintained for approximately 100 days on a caries-producing diet, although the difference is less striking when the animals

² Whole wheat flour, 30%; whole powdered milk, 30%; cornstarch, 20%; confectioners' sugar, 15%; alfalfa meal, 4%; and sodium chloride, 1%.

are maintained for longer periods of time. It is of interest to combine the male and female animals in the control and ingestion groups. With more animals in each group, the difference between the mean caries scores seems more significant (i.e., below the .01 level). These data strongly indicate that the animals employed enjoyed a reduction in caries experience associated with the ingestion of the ammoniated products studied.

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Chloromycetin in the Treatment of "Red Leg"

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The epidemic disease "red leg" has long been a source of difficulty to workers using any of the usual species of frogs as experimental animals. Control of the disease has always been unsatisfactory, as far as can be ascertained from the literature. It was therefore considered worth while to report the use of chloromycetin (chloramphenicol) in the successful treatment of the homologue of frog red leg in a toad, *Bufo marinus*.

The disease was apparently first described by Ernst (6), who isolated a bacterium, which he named *Bacillus ranicida*, from infected frogs. Sanarelli (3) studied an organism, which he isolated from frogs with red leg and named it *B. hydrophilus fuscus*. He reported the organism to be pathogenic for a variety of anurans and urodeles and briefly described some of the pathological findings in infected frogs. Russell (8) reported the pathology of the infection in more detail and described the experimental infectivity of the organism for some of the common laboratory mammals when injected intravenously; he also mentioned the production by the organism of at least two toxins that had pronounced effects upon frog cardiac and skeletal muscle and central nervous system. The nomenclature was revised to *Proteus hydrophilus* in the fifth edition of Bergey's Manual (1) in 1939. In the sixth edition (3) it was changed to *Pseudomonas hydrophila*. In 1942 Kulp and Borden (7) made an extensive study of the bacteriology of the disease, describing two distinct strains of the organism from different sources and reviewing the pathology in frogs. They indicated the existence of "carrier" frogs that harbor the organism in their gall bladders.

The disease in anurans is septicemic and is remarkable

¹ This work was aided by grants from the Ella Sachs Plots Foundation and the Continuing Research Fund of the University of Colorado School of Medicine.

for its virulence and fulminating course. All organ systems are involved, more or less, by the occurrence of widespread petechial hemorrhages and/or septic thrombi, as well as disseminated miliary abscesses. In frogs the skin of the legs becomes markedly hyperemic and hemorrhagic. Death is the usual termination of the disease.

During the course of studies in this laboratory on *Bufo marinus*, three specimens of native toads (*B. boreas* and *B. woodhousii*) were introduced into a colony of 30 specimens of *B. marinus* that had been in excellent health in the laboratory for 16 months. Within 10 days the first case of an epidemic disease, which destroyed a third of the colony before it was controlled, appeared. The disease was studied bacteriologically² and pathologically and found to be a classical example of *F. hydrophila* septicemia. Source of the infection was subsequently traced to an infected frog colony with which the native toads had been housed prior to their acquisition by the author.

In view of the reports that chloromycetin (chloramphenicol) is effective *in vitro* (2) against various species of *Proteus* and *Pseudomonas* and in the treatment of human urinary tract infections (4), this drug was tried on the remaining animals in the colony. At the beginning of treatment most of the animals showed the early stigmata of the disease, namely, moderate reddening and capillary dilatation over the lower belly. The dosage was derived empirically from that used in man and other mammals, and set at 5 mg/100 g initially, followed by 3 mg/100 g twice daily for five days. The drug was dissolved in distilled water in concentrations of 5 mg/ml for the initial dose and 3 mg/ml for subsequent doses. Solution of these amounts was readily accomplished (in contradiction to the solubility data given by Woodward [10] by slight warming. The solutions were administered by gastric intubation, a procedure which is easily performed in these animals.

One of the treated animals was in an advanced stage of the disease (signs of extensive central nervous system damage) at the beginning of therapy. This animal survived 4 more days (twice the survival expectancy of untreated animals in advanced stages) but succumbed to what superficially resembled myocardial decompensation in mammals, presumably due to irreversible myocardial damage incurred before inception of therapy. The remainder of the animals responded well. Now, some two months later, they have shown no further evidence of the disease and again are in their former state of excellent health.

These results suggest that colonies of amphibians may be maintained free from infection by *P. hydrophila* by the administration of chloromycetin at the time the colony is established, either in the water in which the animals are kept, or, preferably, in the manner described above. Similar measures might be practiced on new additions to the colony. The latter seems desirable in view of the report by Emerson and Norris (5) that the disease is

²Thanks are due Elizabeth O'Toole, of the Department of Bacteriology of the University of Colorado School of Medicine, with whom a detailed report of the pathology and bacteriology of the infection in *B. marinus* will be published elsewhere.

endemic in many of the natural habitats from which frogs are collected commercially.

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A Diploid Form of *Medicago sativa* L.

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There have been numerous cytological investigations of common alfalfa, *Medicago sativa* L. Reviews by Fryer (2) and Senn (4) show that the normal chromosome number in somatic tissues is 32. Fryer reported one exceptional plant as having 35 chromosomes. Other abnormal numbers were 31 and 33, found by Skovsted (5) in a study of twin seedlings. The work of Ledingham (3) strongly suggests that the species originated as an autotetraploid, and Tysdal et al. (7) have concluded that at least certain genetic data could be interpreted on the basis of tetrasomic ratios rather than the commonly used disomic approach. If *M. sativa* has an autotetraploid origin, it is to be expected that fertile 16-chromosome forms would exist. So far as the authors are aware, no such forms have been reported. It is the purpose of this paper to record what appears to be a 16-chromosome form of *M. sativa* and to present preliminary data on its cytology and breeding behavior.

In 1947 a small sample of seed labeled *Medicago sativa* was received from Russia through H. A. Senn, senior botanist, Division of Botany and Plant Pathology, Science Service, Ottawa. It was given the Saskatoon accession number S-2128. According to information accompanying the sample, it came from the Botanical Gardens, Academy of Sciences, Armenian S.S.R. at Erevan, Kanaku, U.S.S.R., and was collected from one wild-growing plant. Forty-three seedlings were established in the field nursery at Saskatoon in June, 1949.

The plants started to flower in early August, and it was then apparent that S-2128 was not a normal form of *M. sativa*. It was similar to the latter in having purple flower color, with no trace of yellow present. It was similar also in that a later examination showed the pods to have up to 3 or 4 coils and the relative lack of pubescence common to *M. sativa*. In various characters, how-

ever, such as size of flowers, pods, seeds, leaflets, and fineness of stem, it was much more like 16-chromosome forms of *M. falcata* than normal *M. sativa*. It exhibited also the decumbent growth habit common to 16-chromosome forms of *M. falcata*.

Root-tip smears were made from several plants of S-2128 and were compared to similar material from typical plants of *M. sativa*. The technique followed was based upon that described by Smith (6). Living root tips were first placed in a saturated solution of paradichlorobenzene for 30-60 min to shorten the chromosomes before being killed and were left for 24 hr in a 3:1 solution of 95% ethyl alcohol and glacial acetic acid. They were then softened for 3-5 min in a 1:1 solution of 95% ethyl alcohol and concentrated HCl, and returned to the alcohol-acetic acid solution for at least 3 min before staining with acetocarmine.

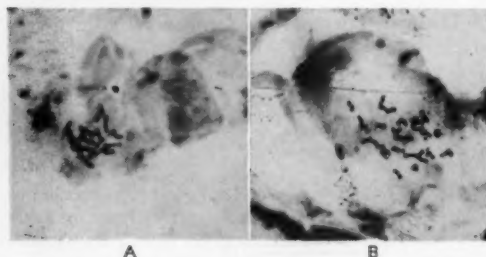


FIG. 1. A, somatic chromosomes from S-2128, magnification approximately $\times 1,300$. B, somatic chromosomes from typical plant of *M. sativa*, approximately $\times 1,300$.

Fig. 1 A is representative of a large number of determinations from root-tip cells of S-2128. The chromosome number is definitely 16. Cell size and chromosome length were not determined, and any apparent differences in length of the chromosomes, based upon A or B in Fig. 1, would not necessarily represent true differences, because the two preparations were not consistently left in the shortening solution for exactly the same duration. Even if this had been the case, it is possible that the cells of different root tips might react unequally to the paradichlorobenzene. In his study of *M. sativa* and *M. media*, Fryer (2) did not observe chromosomes with satellites. In Fig. 1 B there are four chromosomes, each with what appears to be a satellite. Similar formations involving two chromosomes have been observed in S-2128 and show faintly at the lower left-hand corner of the field in Fig. 1 A. Although these structures have been noted in other cells, it is felt that further studies including meiotic divisions are necessary in order to establish definitely the presence of satellites. If these observations are confirmed they would afford good supporting evidence for the autotetraploid origin of *M. sativa*.

In addition to the cytological data presented above, data were obtained also from a series of crosses involving S-2128 as the pistillate parent. All crosses were made August 11 and harvested September 17. Ordinarily the time allowed was sufficient for seed to mature. The period was near the end of the normal seed-setting

season, however, and temperatures were lower than usual. As a result, some of the seed harvested, particularly from selfing and intercrossing, was not fully matured. The flowers were not emasculated for selfing or intercrossing. All flowers outcrossed were emasculated by clipping the standard, tripping the staminal column, and then blowing off the pollen. The data obtained are summarized in Table 1.

TABLE 1
SUMMARY OF SELFING AND CROSSING RESULTS

Parents	No. of flowers pollinated	No. of pods set	No. of seeds	Seeds per flower	Seeds per pod
S-2128	226	20	25	0.11	1.25
S-2128 intercrossed	341	275	1278	3.75	4.65
S-2128 \times <i>M. sativa</i> (32-chromosome form)	149	45	21	0.14	0.47
S-2128 \times <i>M. falcata</i> (32-chromosome form)	53	21	6	0.11	0.30
S-2128 \times <i>M. falcata</i> (16-chromosome form)	147	115	603	4.10	5.24

The selfing results suggest that S-2128 is rather highly self-sterile. The 226 flowers self-pollinated (Table 1) represented 11 plants. The 20 pods and 25 seeds harvested represented only 120 flowers from 4 plants, however. The remaining 106 flowers from 7 other plants proved to be completely self-sterile. The intercrosses show fairly normal cross-fertility, as compared with the average of 5.54 seeds per pod found previously (1) in a study of 32-chromosome *M. sativa*. Bolton (1) has shown that intercrosses between related plants set fewer seeds per flower, and that the progenies of these crosses are lower in seed and forage yield than comparable outcrosses. As previously noted, the information accompanying the original sample of S-2128 stated that the seed had been collected from one plant. That statement is supported by the results of selfing and intercrossing, as well as by the relative uniformity of the progeny for morphological characters.

When S-2128 was outcrossed to 32-chromosome forms, the results were similar whether *M. sativa* or *M. falcata* was the staminate parent. The number of seeds per flower was similar to that from selfing, but many of the pods contained only aborted seeds, which were not included in the calculations of total seeds harvested. These results compare closely with unpublished results obtained at Saskatoon, where it was found that normal seed-setting occurred in crosses between 32-chromosome forms of *M. falcata* and *M. sativa*, but that, when pollen from 32-chromosome *M. sativa* was applied to the stigmas of 16-chromosome *M. falcata*, although a fair percentage of the crossed flowers set pods, the pods contained only aborted seeds. The few seeds obtained from S-2128 \times 32-chromosome forms have not been tested. They may prove to be selfs, although this is unlikely, both because the flowers were emasculated, and because some of the plants involved set no seed when selfed. It is more likely that they will prove to be triploids or induced tetraploids, such as those reported by Ledingham (3) in crosses between

16-chromosome *M. falcata* and 32-chromosome *M. sativa*.

To summarize, it may be said that a 16-chromosome form of *M. sativa* has been found. This form is highly self-sterile and highly cross-sterile when crossed to 32-chromosome forms of *M. sativa* and *M. falcata*. It shows normal cross-fertility when crossed to 16-chromosome forms of *M. falcata*.

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Quantitative Aspects of the Action of Insulin on the Glucose and Potassium Metabolism of the Isolated Rat Diaphragm

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In a previous communication (3) experiments were described from which it was concluded that the utilization of glucose by the isolated rat diaphragm is associated with a shift of potassium from the medium into the tissue, and that both glucose utilization and potassium shift are increased by the addition of insulin. In order to study the quantitative aspects of these reactions, experiments were carried out with varying concentrations of insulin.

The technique was different from that used previously. Rats of 80–100-g body weight were decapitated after fasting for 24 hr; their diaphragms were removed and cut into quarters. The quarters were kept in ice-cold buffer solution (2) before the actual experiment started. Eight quarter-diaphragms, representing one-half of the left and right hemidiaphragms of 4 rats, were transferred to a flask containing 2 ml of buffer solution in which 200 mg % of glucose had been dissolved. After equilibration with a gas mixture containing 93% O₂ and 7% CO₂, the buffer-diaphragm system was incubated for 1 hr at 37° C, with shaking at a rate of 120/min. The remaining 8 quarter-diaphragms of the same rats were incubated in a buffer-glucose solution of the same composition that contained, in addition, insulin in the concentration to be tested.

This arrangement was chosen so as to make the conditions under which the diaphragms were incubated as similar as possible, with the only difference that insulin was present in one flask and absent from the control flask.

¹ Holding a grant from the Organon Laboratories, Inc., Oss, Netherlands.

After 1 hr the flasks were cooled and the contents centrifuged. Glucose (5) and potassium (7) concentrations in the medium were determined, and the diaphragms were weighed after blotting on filter paper. The difference (Δ glucose) between the quantities of glucose (calculated as mg/100 mg wet tissue) that have disappeared from the medium in the flask with insulin and that without insulin is the effect of the added insulin on glucose utilization.

While studying the changes in potassium content of the medium in these incubation experiments, it was found that, with the use of quarter-diaphragms, the potassium content of the medium increased as a rule, whereas it usually decreased when hemidiaphragms were used. Apparently a diffusion of potassium out of the "surviving" tissue takes place more rapidly from quarter-diaphragms than from hemidiaphragms. This is easily understandable because of the greater damage to the tissue that takes place when the diaphragm is divided into 4 parts.

In the present series of experiments the potassium content of the medium increased in both flasks, but in the vessel containing insulin the increase was invariably less than in the one without insulin. The difference (ΔK) between the increase of the potassium content (calculated as microequivalents per 100 mg wet tissue) of the medium in the flask with insulin and that without insulin is the effect of the added insulin on the potassium shift, associated with increased utilization of glucose by the tissue.

The glucose and potassium effects of increasing concentrations of insulin were plotted in a curve (Fig. 1). The

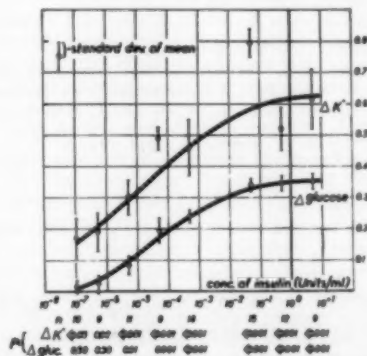


FIG. 1. Effect of varying concentrations of insulin upon glucose utilization (mg/100 mg wet tissue) and potassium shift (microequivalents/100 mg wet tissue) in experiments with isolated rat diaphragms; n = number of experiments; P = Fisher's value of probability.

graph illustrates that, with augmenting concentrations of insulin, both glucose and potassium effects increased until, at a level of about 10^{-2} – 10^1 units/ml, both effects tended to become more or less constant. In the region of lower concentrations of insulin, it was found that 10^{-2} units/ml still had a significant effect upon the potassium shift, whereas the effect upon the glucose utilization was no longer detectable. In general, the figures for the effect of insulin on potassium shift were more irregular than

those for the effect on glucose utilization; this demonstrated itself in the relatively large standard deviations of ΔK compared with those of Δ glucose. The greater irregularity of the potassium shift may be due to the varying degree of tissue damage at the excision and the ensuing leakage of potassium from the injured muscle fibers.

Extremely small concentrations of insulin can be detected by this method. Even with insulin concentrations as low as 5×10^{-3} units/ml, significant effects on the glucose and potassium metabolism of the isolated rat diaphragm were observed. Lower concentrations appeared to have a potassium effect, but the level of significance was less than 1%. Apparently this technique makes it possible to detect insulin in amounts that are much smaller than those reported by previous observers (1, 6). It should be borne in mind, however, that variations in sensitivity of the diaphragms may occur.

The sample of pure insulin used in these experiments contained 28 units/mg.² The smallest concentration of insulin that still produced a significant increase in glucose utilization was 5×10^{-6} units/ml, which, assuming a molecular weight of insulin of 48,000, amounts to 4.5×10^9 molecules per flask. This number of insulin molecules enabled the diaphragm to utilize about 10^{18} more molecules of glucose than diaphragms in the control flask without insulin. This extra utilization of glucose was associated with an extra shift of about half as many atoms of potassium from the medium into the tissue. With further refinements of technique, the isolated diaphragm test may offer opportunities for the determination of minute amounts of insulin.

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² We are indebted to J. Lens (4) for a gift of pure insulin.

A Method for Silver Staining of Nerve Fibers in Whole-Mount Preparations of Blood Vessels

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Humphreys (3, 4) demonstrated vascular nerve fibers on cerebral blood vessels, using a modification of the Bodian (1) technique. He obtained better and more consistent results than with either the modified Gros-Bielschowsky technique of Penfield (5) or Huber's (2) methylene blue technique.

On cerebral blood vessels of the pia mater we have ob-

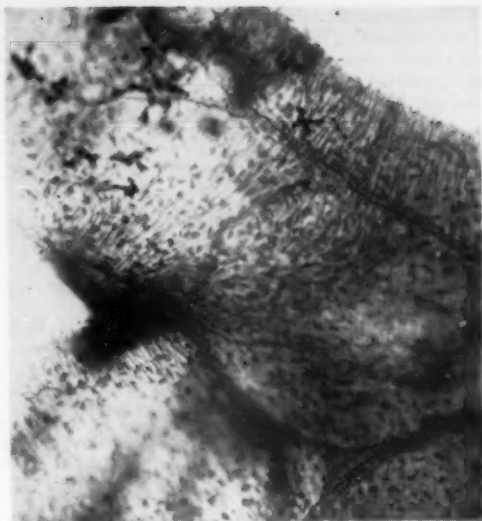


FIG. 1. Arterial branch of vertebral artery showing the crossing and branching of large mixed fiber bundles of the adventitia. The dark stellate cells are chromatophores. Objective, 16 mm; ocular, 10 \times . Protargol.



FIG. 2. Group of myelinated fibers becoming related to a branch of the basilar artery. Objective, 16 mm; ocular, 10 \times . Protargol.

tained more satisfactory results by use of the following modification of the Bodian technique. Blood vessels of anesthetized animals were flushed with a physiological salt solution, followed by perfusion with neutral 10% formalin. The leptomeninges containing the blood vessels were dissected from the brain and brain stem, and pinned flat on paraffin for 24 hr in a Petri dish contain-

ing 10% formalin. The tissues were then washed in water, dehydrated in alcohol, and cleared in cedarwood oil. After clearing, they were hydrated and placed in distilled water for 1-2 hr. This procedure seemed to give the tissues a greater clarity and stainability. The connective tissue elements took less silver stain, and small nerve fibers appeared finer and darker. Individual types of fibers could be distinguished in the roots of cranial nerves that lay in the pia-arachnoid. Subsequently the standard Bodian (1) technique for staining paraffin sections was employed. The length of time the tissues were left in the various solutions varied with the tissue under study and was determined by direct observation. In general, they were left in the Protargol solution for 32-48 hr, and in the other solutions for approximately the same period as suggested by Bodian for section-staining. It was necessary, however, to flush the solutions from within the vessels each time a change was made. This was easily accomplished by gentle pressure on the blood vessels exerted with a round-tipped glass rod. After staining, the tissues were dehydrated and cleared in cedarwood oil and xylene. Mounting in clarite and permount proved satisfactory, and no fading was seen after 6 months.

The technique consistently demonstrated the nerve fibers along blood vessels. Small and large fibers, singly or in bundles, were readily distinguished (Figs. 1, 2). These preparations made it possible to follow nerve fibers along blood vessels for relatively long distances.

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Molluscan Shells as a Practical Source of Uroporphyrin I

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To obtain a supply of uroporphyrins for laboratory work has always presented difficulties. Human cases of porphyria have been the main source, but large quantities of urine must be handled to extract the uroporphyrin, and the cases themselves are uncommon. Extraction from urine by the simplified method of Sveinsson, Rimington, and Barnes (6) considerably shortens the procedure, but where large amounts of uroporphyrin are needed an alternative source is most desirable. Possible animal sources (the bones of *Sciurus*; the feathers of *Turacus* which yield turacina) involve scarce and expensive material. Very large amounts of uroporphyrin in an easily manageable form have, however, been found in molluscan shells.

Shell porphyrins were originally recognized by Fischer and Jordan (3), and have since been extracted by several

workers (Fischer and Haarer [2], Waldenström [9], and Tixier [7, 8]). The supposed pentacarboxylic conchoporphyrin of Fischer and Jordan was found by Nicholas and Comfort (5) to be a mixture of uro- and coproporphyrin. In most of the porphyrin-containing forms, uroporphyrin I occurs in an almost pure state. Many species are suitable for use as a source: Porphyrins have been shown to occur only in marine genera, and in largest quantity among the pearl oysters (1). The most convenient material for large-scale extraction is the Persian lingah oyster (*Pinctada vulgaris*), which is an article of commerce, and may yield as much as 10 mg per g of shell. *Trochus niloticus*, also used in the button industry, is an inferior source, owing to the thickness of the shell and the large amount of unpigmented mother-of-pearl.

The technique described by Tixier (7), in which shells are extracted with methanolic HCl to esterify the pigment, has the drawback of expense, since several liters of solvent are required per kg of shell. The following technique gives satisfactory results.

Shells of *Pinctada vulgaris* are selected under the ultraviolet lamp for their porphyrin content. They are coarsely broken, and the powder is added in small amounts to concentrated aqueous HCl in a very large beaker, allowing 500 ml of solvent to each 50 g of shell. Octanol and other antifoam agents should not be added, since these contaminate the product. The extraction is left to proceed at room temperature for 24 hr, the mixture brought to pH 1 approximately, and the debris removed by filtering over glass wool.

A chromatographic column is prepared by shaking pure acid-washed talc with N HCl, and sedimenting it over suction in a large tube (2.5-in. diameter) plugged with cotton wool. The filtered porphyrin solution is passed through this column by suction, and the chromatogram washed with 20% acetone in tap water. Partial development takes place, with separation of red, blue, and violet nonporphyrin bands. The extent of the porphyrin zone is checked by fluoroscopy. Red fluorescence in the subsidiary blue band should be ignored, as it appears to be due to an unidentified nonporphyrin pigment (1).

The column is extruded and the main porphyrin zone extracted with acetone containing 10% concentrated aqueous HCl. The filtered extract is concentrated *in vacuo* until it no longer smells of acetone, then diluted with an equal volume of distilled water, and adjusted to pH 3.2 by addition of saturated sodium acetate solution until precipitation of the uroporphyrin occurs. It is left overnight in the ice chest, the precipitate collected on a sintered glass filter, redissolved in concentrated aqueous HCl, and reprecipitated by neutralization. The final precipitate is then dried, esterified in a small volume of methanolic HCl, transferred to chloroform by dilution with water, chromatographed once or twice on alumina, and crystallized from chloroform-ethyl acetate mixture. The yield is approximately 1 mg of ester per g of shell.

The homogeneity of the sample should be checked by paper chromatography, using the method of Nicholas and Rimington (4). Uroporphyrin III has not been detected so far in molluscan shells.

Other species of *Pinctada* and *Pteria*, as well as of *Placuna*, *Malleus*, and *Pinna*, vary widely in their suitability, and in the quantity of porphyrin and nonporphyrin pigment present. The common pearl oyster (*Pinctada margaritifera*) is unsuitable. Of American species, *Pteria radiata* Lam. is a relatively good source, and smaller amounts can be obtained from many Trochidae and from *Trivia* spp. The only form containing amounts of porphyrin comparable to those in *Pinctada vulgaris* is the relatively unobtainable *Cypraea mappa*, the nature of whose porphyrin has not been established.

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Recent Patterns of Employment and Unemployment

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The adoption of the federal old-age insurance law in 1935, and the passage of state unemployment insurance laws about the same time, created a new and rich source of data on employment and unemployment in this country.

Since 1937, a vast amount of detailed information on the employment and unemployment experience of American workers has been collected as a by-product of the operations of these social insurance programs. The brief span of 12 years since 1937 has been strongly affected by World War II. However, the employment data now reported regularly by every covered firm, and the unemployment information supplied by jobless workers claiming unemployment benefits, have already revealed certain patterns and relationships. This paper will discuss briefly some of the patterns of employment and unemployment observed thus far.

A brief description of the information available may be helpful as a background. (A more detailed description is given in [2].) The federal old-age insurance law covers about two-thirds of all employment in this country. The following groups are excluded: government workers, self-employed persons, railroad employees, agricultural workers, persons engaged in domestic service, and employees of nonprofit-making religious, educational, and charitable organizations. The New York Unemployment

Insurance Law, which will be used as an example of the state programs, includes the same industries as the federal act but covers only firms with 4 or more employees, thus excluding workers in small firms with 1, 2, or 3 employees.

All covered employers report quarterly the earnings during each calendar quarter for each individual who was employed at any time during this period. Individual key-punch cards are then prepared for each person and sorted by social security number to give a complete earnings record for each worker. In addition, employers report certain total figures, such as the number of persons employed during the week ending nearest the 15th of each month in the quarter. These reports are coded by industry and geographic area and summarized to provide comprehensive data on employment.

One simple employment pattern revealed by these data is the average number of weeks of work per person in a calendar year. This can be derived as follows: In the state of New York, for the year 1939, the number of persons working in the midweek of each of the 12 months was obtained by calculating the data from the employer reports. The average of these 12 numbers was 3,114,000. Studies of weekly employment figures of individual firms for all 52 weeks of the year have shown that the average of the 12 mid-week-of-the-month figures closely approximates the average weekly employment for the year. Thus, the average weekly covered employment in the state in 1939 may be estimated at 3,114,000.

The total man-weeks of work during 1939 can be derived as $52 \times 3,114,000$, or about 162 million man-weeks. A count of the number of different workers with one or more quarterly wage cards showed that 4,450,000 different persons had worked in covered employment during 1939. There were 162 million man-weeks spread among 4,450,000 persons, or an average of 36.4 weeks of work per person.

In New York State, during the 10 years from 1939 to 1948, average employment rose from 3,114,000 in 1939 to 4,366,000 in 1948, and the number of individual workers during a calendar year rose from 4,450,000 in 1939 to 6,350,000 in 1948. Yet the average number of weeks of work per person showed little variation.

During the 3 prewar years, 1939-41, the range was from 36.0 to 36.4. During the 4 wartime years, 1942-45, the average number of weeks worked per person ranged from 34.2 to 34.6. The 3 postwar years, 1946-48, showed a range from 35.5 to 35.8. For the entire 10-year period, the range was from 34.2 weeks in 1942 to 36.4 weeks in 1939. The relative stability of this average is noteworthy in view of the dynamic changes in employment resulting from World War II and the sharp rise in both the level of employment and the number of persons in the labor market.

A similar analysis applied to data on employment covered by the old-age insurance law recently published by the federal Bureau of Old-Age and Survivors Insurance (4) showed 36.1 weeks of work per person in 1947 and 37.2 weeks in 1948.

A second type of analysis uses the employment data arising out of the long-range, continuous-work-history

study that has been carried on since 1937 by the federal Bureau of Old-Age and Survivors Insurance. A similar long-range study, which includes both employment and unemployment experience, has been undertaken in New York State using the records of the Division of Placement and Unemployment Insurance. It should be noted that, before the social insurance programs began in 1937, it was difficult and expensive to collect any data on individual employment experience over a period of many years. (One study, covering the experience of several thousand boys and girls from 1921 to 1939, has been made by Thorndike and Lorge [1, 3]).

The old-age insurance study at present covers a 1% random sample of all persons who have received social security account numbers since 1937. This is done by selecting certain end digits of the 9-digit social security numbers. At the end of 1945, this sample totaled about 750,000 persons. It is planned to expand the sample at some later time to include about 2.5% of the covered workers. The New York sample is the approximately 10% group of persons who have 2 as the sixth digit of their social security numbers. The two studies purposely overlap, so that the New York data on unemployment and the old-age insurance data on employment in small firms with fewer than 4 workers can be combined to give a more complete picture. For each person the available data from 1937 on show age, sex, industry of each employer, calendar quarters and years of work, quarterly and annual earnings, and the geographic area of employment. The unemployment data available for the New York group show the number of weeks of unemployment each year as represented by claims for unemployment insurance.

Detailed tabulations of the old-age insurance materials are published annually in the *Handbook of Old-Age and Survivors Insurance Statistics*. The following figures are derived from the 1945 *Handbook*.

The 1% continuous-work-history sample covering the 9 years from 1937 through 1945 includes 755,000 persons who, in one or more years during this period, were employed in covered industries. Almost one-fifth of these persons had been employed in all 9 years. There were 463,000 men in the sample, or 62%; 24% of these men had been employed in all 9 years. Of the 287,000 women in the group, only 10.6% had employment in all 9 years.

Another view of the 9-year pattern of employment can be obtained by considering the 329,500 persons in the sample who had employment in 1937, the first year of the old-age insurance program. Of these 329,500 persons who were working in 1937, 143,000 or 43.4%, have worked in each of the 9 years from 1937 through 1945. Again the men showed a greater stability of employment, with 47% of the 1937 workers having some employment in each of the 9 years, as against 34% for the women.

The 1945 *Handbook* also contains data on the distribution of the workers in the 1% sample into groups having continuous patterns of 1-9 years of employment, and those having intermittent patterns of 2-8 years of employment. Some of the other tables give the following information:

1. The number of persons beginning their employment history in each year.
2. The number of persons having their latest employment in each year.
3. A cross-tabulation of the workers showing the number of years, and the latest year, of employment.
4. A cross-tabulation of the workers by number of calendar quarters of employment from 1937 to 1945, and the number of years of employment.

Similar data are given in the recently published 1946 *Handbook*, which covers the 10-year period from 1937 to 1946. One interesting 10-year result may be mentioned: In 1937, there were 33 million persons in this country employed in firms covered by the old-age insurance law; 40% of these worked in covered employment in all the 10 years from 1937 to 1946.

In New York State sample studies have shown that, in 1943 (using this wartime year of high-level employment as an illustration), 72% of the workers had one employer during the year; 15% had two employers; and 13% had three or more employers. In the same year, 78% of the workers were employed in one industry; 15% had employment in two industries; and 7% worked in three or more industries. The industry code used has about 60 different classifications.

The unemployment data in New York have revealed an invariant relationship in the duration of unemployment in every year. Unemployment benefits in New York are payable for a maximum of 26 weeks in the 12-month "benefit year," which begins in June. The distribution of persons by the number of weeks of benefits shows remarkable regularity for each year. The ratio between the cumulative number of persons who have received at least x weekly checks and the group of persons who have received at least $x+1$ weekly checks is a constant for all pertinent values of x . In other words, the probability of persons with x weeks of unemployment reaching at least $x+1$ weeks is a constant for x ranging in value from 1 to 25. For example, in the 1946-47 benefit year, this constant probability was .94, with the smallest ratio, .933, occurring when x was taken as 1, and the largest ratio, .945, occurring when x was taken as 15 weeks.

In all the 9 benefit years from 1940-41 through 1948-49, this invariant relationship was found. In 6 of these years the constant probability was approximately .94. In 1942-43 the constant was .92. In 1943-44 and in 1944-45 it was .91. In considering the extremely small variation in this ratio within each year and the minor change from .94 to .91 during the height of wartime activity, it should be noted that the period from 1940 to 1949 varied from considerable compensable unemployment in 1940-41, when 830,000 persons received benefits, to very little unemployment in 1944-45, when only 76,000 persons received benefits. It then rose again in the post-war period, with approximately 900,000 persons receiving benefits in 1948-49. This constant ratio existed in spite of the wide variations in economic conditions.

Another feature is the incidence of recurrent beneficiaries, persons who receive unemployment insurance

benefits in two or more successive years. For example, about 900,000 persons received one or more checks during the first year of the program in New York, in 1938. Of this group, 44% also received benefits in the second year; 24% received benefits in the third year; and 12% received benefits for four successive years. The tendency for about half the beneficiaries in any one year to receive unemployment benefits also in the following year has been observed throughout the period from 1938 to 1949.

Some other patterns may be mentioned: From the data on unemployment, it is possible to obtain the distribution of the persons claiming benefits by the number of periods of unemployment in each year. Taking the year 1939 as an example, there were over 900,000 persons who filed claims for benefits. About 55% of these persons had only one period of unemployment during this year, 25% had two, 10% had three, and 10% had four or more.

Intensive analysis of the information being produced

as by-products of the social insurance programs and additional studies starting from the clues provided by these data will be necessary to test the stability of the patterns that are being revealed, as well as to discover the significant relationships that may be hidden in the data available thus far. Such studies may lead to the development of a comprehensive theory to coordinate and explain the observed regularities.

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Comments and Communications

On the Persistence of 2,4-D in Plant Tissue

In a recent issue of *SCIENCE*, Tullis and Davis (1950, **111**, 90) have interestingly reported characteristic symptoms of 2,4-D injury during the season of 1949 on developing shoots of Chinese tallow trees (*Stillingia sebifera* Michx.) that had been subjected to 2,4-D in 1948 but not in 1949. Further, they observed no injury during the season of 1949 on chinaberry trees (*Melia Azedarach* L.) that had been severely injured in 1948 by 2,4-D. They have interpreted these results to indicate the persistence of 2,4-D in tissues of the Chinese tallow tree from one growing season to the next, and the lack of persistence in the tissues of the chinaberry tree under similar conditions. The facts are of special interest because they contribute to an understanding of the mode of action of 2,4-D.

The appearance of 2,4-D injury in perennial plants the season following treatment is, however, not uncommon. Many instances are on record of orchards and vineyards treated during one season with formulations of 2,4-D for weed control or for the prevention of premature abscission of fruit, which developed anomalous leaves, flowers, and fruits the following season (Bryant, L. R., Vincent, C. L., and Schafer, E. G. [*Proc. Amer. Soc. Hort. Sci.*, 1947, **49**, 63]; Harley, C. P., Moon, H. H., and Regeimbal, L. O. [*Proc. Amer. Soc. Hort. Sci.*, 1947, **50**, 38]; Marsh, R. S., and Taylor, C. F. [*Proc. Amer. Soc. Hort. Sci.*, 1947, **49**, 59]; Moon, H. H., Regeimbal, L. O., and Harley, D. P. [*Proc. Amer. Soc. Hort. Sci.*, 1948, **48**, 81]; and Teske, A. H., and Overholser, E. L. [*Virginia Fruit*, 1947, **35**, 15]). The responses are variously described as delayed foliation, malformed and stunted leaves, fruits with oblong shape and open core, double fruits, and fruits with only rudimentary seeds.

Also, an experience has been reported by Tukey and Hamner (*Proc. Amer. Soc. Hort. Sci.*, 1949, **49**, 95) in a mixed planting of sweet and sour cherry trees (*Prunus avium* L. and *P. cerasus* L.), which was sprayed the fall of 1946 with a mixture of naphthalene acetic acid and 2,4-D. The following season many leaves were dwarfish, narrow, and sharply serrate; both pits and fruits were markedly elongate and pointed; receptacles were much enlarged; strong vascular development occurred in both fruit and pedicel; flesh was strongly adherent to the pit; and chemical composition of the fruit was altered. However, repeated applications of various growth regulators to cherry trees by Tukey (unpublished data) in the spring and midsummer produced no such visible effects the following season.

The facts suggest that there are critical or sensitive periods in the growth of the cherry, and that applications of growth regulators made in the fall may produce a striking effect upon carpel development, which may in turn be reflected in the developing fruit the following season. Applications made at other times may, however, fail to produce a response, because critical parts may be already formed, not yet formed, or in a state of physiological insensitivity or inactivity.

Studies by D. P. Watson (*Amer. J. Bot.*, 1948, **35**, 543) on modification of bean leaves as a result of treatment with 2,4-D bear on this point. They show delayed expression of the effect of growth regulators, associated with the stage of development of a leaf at the time the treatment is made. Watson concludes: "Frequently, plants that exhibit what appears to be delayed injury have received leaf injury during the formation of buds which did not expand until some time later."

Similarly, Arthur J. Eames (*Amer. J. Bot.*, 1949, **36**, 571), working with nut grass (*Cyperus rotundus* L.)

has concluded: "The opinion that in many plants the stimulus of various growth-regulating substances continues for various periods of time is probably based on (1) the continuing activity of the abnormal meristems; and/or (2) the development, long after treatment, of dormant buds injured (while growing) before dormancy. New tissues and organs formed after treatment are not affected."

Taken all together, the present information suggests that distinctions must be made between (a) persistence of 2,4-D in plant tissues, and (b) delay in visible expression of effects of 2,4-D.

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Concept of Complementarities

In the interest of accuracy and fairness, the following remarks aim to correct erroneous impressions given by the historical introduction to the interesting paper of A. M. Schechtman and T. Nishihara in *SCIENCE*, April 7, 1950.

Four years prior to the publication of the paper of Breinl and Haurowitz (1930), I had advanced the concept of antibodies as units complementary to their antigens in addresses before the American Chemical Society and elsewhere. In these talks a coin was used to illustrate the antigen surface, and a piece of tin foil pressed against it formed the specific reverse pattern, illustrating the specific antibody. I pointed out that the top surface of the foil, away from the coin, formed a duplicate of the coin surface, illustrating reproduction at the molecular or near-molecular level of structure. Since some years of public and private discussion developed no objection or alternative view of antibody formation, I sent a paper to an American scientific journal briefly outlining the view. After some consideration, the paper was rejected. It was then sent to another American journal, whose editor, to justify his refusal to publish it, showed me the letter of a prominent "referee," who wrote "there are an infinite number of similar speculations possible." The paper, entitled "Some Intracellular Aspects of Life and Disease," was finally sent to *Protoplasma*, which published it (1931, 14, 296), with illustrations much like those of Schechtman and Nishihara, except that the latter include the later, more detailed concepts of Linus Pauling.

My *Protoplasma* paper was reviewed in an editorial by Stephen Miall in *Chemistry and Industry* (London, 1932), in which he used the apt engineering term "template" (or templet) to describe the function of the antigen. This term, as well as the coin-foil analogy mentioned above, has become common usage.

Furthermore, "the possibility of applying concept of complementarity to the more general problem of specificity in biological synthesis" had been suggested long before the references quoted by Schechtman and Nishihara; e.g., in a paper by J. Alexander and C. B. Bridges on "Some Physico-chemical Concepts of Life, Mutation, and Evolution" in Vol. II of *Colloid Chemistry* (1928), where still earlier views of Leonard Troland

on catalysis are in part reprinted (see also Alexander and Bridges, *Science*, 1929, 70, 508). Much of the earlier work, with its bearing on embryonic differentiation, is given in *Life, Its Nature and Origin* (1948), by J. Alexander.

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Mr. Alexander's comments on the origin of the idea of complementarity as applied to antigen-antibody relationships will be of interest to persons concerned with the evolution of this line of thought. Our paper (*Science*, 1950, 111, 357) is not, nor was it meant to be, a comprehensive review; the introductory statement concerning the literature was condensed and presented as a minimal background necessary for the exposition of the experiments described. Nevertheless, several recent review papers by Haurowitz, Pauling, and Tyler (references 4, 10, and 12, respectively) were selected for mention to provide more extensive guides to the literature than was possible in the paper. The references provided by Mr. Alexander will doubtless be a welcome addition for future reviewers who may wish to decide whether the essentials of the idea of molecular complementarity as applied to biological synthesis are rightly attributed to Breinl and Haurowitz.

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Our Flat Planet

Nearly 25 years ago, in Spokane, Washington, a highly reputable and very opinionated local businessman issued a defiant challenge to the entire region in which he lived. His local reputation, he felt, had been endangered by several public arguments in which he stoutly and steadfastly maintained, against all opposition and contradictory to much evidence, that the earth was flat. His challenge to the community was climaxed by an ultimatum published in the forum columns of the leading local newspaper, the *Spokesman-Review*. In effect, his ultimatum told his critics to either "prove they were right or shut up." To back his arguments, he announced in the column that he was placing \$1,000 on deposit in the Old National Bank of Spokane and would pay it to any person who could prove that the earth was round.

As long as his mind had to be convinced that the earth was round, his \$1,000 remained entirely safe, and the money remained on deposit in the bank for a number of years. Then he triumphantly announced, again in the forum column of the same newspaper, that—having given everyone a chance to submit proof that the earth was round and everyone having failed—he felt deeply grateful that he had been able to prove so conclusively to the entire world that the earth was flat.

Fortunately, not many were affected by his reasoning. The only bad feature about this incident lies in the fact that he is a strong religious leader. Some of the children

who have heard his belief paraded before the community may become warped by his crooked thinking. By this means, "eranks" are made.

He really does believe that the earth is flat and prob-

ably will until he dies. This man, Warren Latham by name, has never backed down on his untenable claims.

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Book Reviews

Antibiotics: A Survey of Penicillin, Streptomycin, and Other Antimicrobial Substances from Fungi, Actinomycetes, Bacteria, and Plants, Vols. I and II. H. W. Florey, et al. New York-London: Oxford Univ. Press, 1949. 1,774 pp. \$29.75 the set.

A number of comprehensive reviews of the literature on antibiotics and books concerned with penicillin and streptomycin have appeared during the past five years. In most instances the clinical use of these substances has received major emphasis. The present authors, all members of the famous Oxford team that contributed so much to the successful commercial production of penicillin a decade ago, believe previous writers have neglected the historical foundation of the antibiotics and the interrelationship of the chemical, biological, and clinical approaches. Their survey, as given in Volumes I and II, deals for the most part with laboratory investigations; a third volume, now in preparation, will be entitled "Clinical Application of Antibiotics."

The contents are arranged in eleven parts, with the following captions: "Historical Introduction"; "General Experimental Methods"; "Antibiotics from Fungi"; "Antibiotics from Fungi-Substances other than Penicillin"; "Antibiotics from Actinomycetes"; "Antibiotics from Bacteria"; "Antimicrobial Substances from Lichens, Algae and Seed Plants"; "Penicillin"; "Streptomycin"; "The Action of Antibiotics on Bacteria"; and "Conclusions." The authors, well aware that the rapid accumulation of information in this field precludes publication of an up-to-the-minute reference book, have added an appendix Volume II, which includes a list of the known antibiotics and short summaries of papers not mentioned elsewhere in the text.

There are a number of inaccurate statements in the text and references to procedures now obsolete. These will unquestionably be rectified with the appearance of Volume III. These volumes can be highly recommended to chemists, bacteriologists, and clinicians.

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Reviewed in Brief

Research in Medical Science. David E. Green and W. Eugene Knox, Eds. New York: Macmillan, 1950. 492 pp. \$6.50.

The current medical literature, in contrast to that of former years, is replete with contributions by nonmedi-

cally trained investigators. It was firmly believed in days gone by that one must have a medical degree before embarking on a career of research into the mysteries of the disease process. Today specialists in disciplines seemingly far removed from the bedside are making fundamental discoveries in this area. Often their reports are so highly technical that the results of their researches are put into practice without being understood by the clinicians.

The present volume gives in essay form some of the latest developments in medical research. They are non-technical accounts by outstanding representatives in microbiology, immunochemistry, physiology, and allied disciplines. Some idea as to the variety of subjects covered may be gained from the listing of the first and last titles: "Bacteriophages and Their Action on Host Cells"; "Some Biochemical Problems in Sanitary Engineering"—the former article by a biophysicist, the latter by a sanitary engineer.

The book will be of real value to those working in the basic sciences at medical schools, as well as to scientists in general.

The Nature of Natural History. Marston Bates. New York: Scribners, 1950. 309 pp. \$3.50.

This book is a readable survey of general biology, with special emphasis on ecology, which is virtually equivalent to the author's interpretation of the meaning of natural history: "the study of animals and plants . . . of organisms." The first six chapters supply the essential background of classification and a survey of the plant and animal groups, their historical evolution, reproduction, heredity, and development. The next five chapters deal with the relations of individuals to one another and to the environment. There are interesting discussions of biotic communities, partnership and cooperation, parasitism, and individual behavior. The next section deals with the natural history of populations, their behavior, and geographic distribution. Adaptations and the mechanism of evolution are considered in the next two chapters, which are followed by a final group of three chapters: "Natural History and Human Economy"; "The Natural History of Naturalists"; and "Tactics, Strategy, and the Goal," a consideration of the methods of science and natural history in particular. Throughout the book, Marston Bates' broad background and extensive experience in the tropics make his comments illuminating and render his criticisms of the limitations of laboratory science cogent.

News and Notes

Atmospheric Turbulence Discussion

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U. S. Naval Ordnance Laboratory, White Oak, Maryland

A round-table discussion on atmospheric turbulence was held on May 3 in the Naval Ordnance Laboratory at White Oak, Md. The purpose of the discussion was to establish a contact among scientists, as well as among laboratories, interested in the development of the basic knowledge in atmospheric turbulence.

Among those present were: G. W. Brier, H. B. Byers, K. L. Calder, P. E. Church, P. Donely, E. G. Droessler, H. L. Dryden, F. N. Frenkiel, J. Gerhardt, Mrs. K. Hafstad, E. W. Hewson, Col. B. C. Holzman, A. M. Kuethe, H. Lettau, R. B. Montgomery, H. M. Mott-Smith, Comdr. L. G. Pooler, H. F. Poppendick, G. B. Schubauer, M. E. Smith, B. L. Snavely, V. E. Suomi, O. G. Sutton, and H. Wexler.

Atmospheric turbulence was described as the irregular fluctuations of physical quantities (such as velocity, pressure, and temperature) in functions of both time and space. These fluctuations cover a wide range of scales from very small eddies to the general circulation of the atmosphere.

It was emphasized that the great development of the statistical theory of turbulence and of experimental techniques used by the fluid dynamicists opens new possibilities in the study of atmospheric turbulence. There was general agreement that coordination in the work on atmospheric turbulence is desirable. More particularly, there seems to be a need to use the same scientific language in the treatment of this subject.

The work done and planned in various universities and government laboratories was described and discussed by the participants. The lack of data concerning atmospheric turbulence is obvious, and there is a need for further development of instruments measuring turbulence characteristics. It was recommended that one or more model instruments having clearly defined characteristics

be chosen and used to measure turbulence at a number of meteorological stations.

Information on atmospheric turbulence is needed for various applications. Some scientists interested in their own applications use their particular scientific language, and their ideas and results fail to come to the attention of their colleagues. The two books on the subject (the authors of both books, H. Lettau and O. G. Sutton, attended the round-table discussion) are valuable in the coordination of these results, but there is also an urgent need to coordinate the research itself.

Some of the applications of atmospheric turbulence were discussed at the meeting. Reference was made to the twofold effect of turbulence on aircraft in flight. Small-scale turbulence influences the nature of the flow by acting on the transition of the laminar boundary layer in a turbulent boundary layer, and the macroscopic turbulence is of importance in the stability and the maneuverability of an airplane. The nature and magnitude of gusts that may be encountered in flight have to be taken into account in the structure of aircraft.

There is much concern in the correlation between atmospheric turbulence and the scattering and propagation of microwaves. The need for more information on this subject is felt by the radar scientists.

Turbulence both near the ground and in the upper atmosphere is of great interest. The extension of theoretical and experimental work is needed in such matters as air-sea interchange problems, evaporation, and convection. The role of turbulence in the diffusion of air pollutants, as well as the other fundamental meteorological quantities such as moisture and heat, has to be more fully investigated.

The round-table discussion was organized by F. N. Frenkiel (chairman), E. G. Droessler, and H. Wexler.

About People

Hamilton Anderson, professor of pharmacology and chairman of the department at the University of California School of Medicine, San Francisco, will serve as dean of the Medical School of the American University of Beirut, Lebanon, during the academic year 1950-51. During his stay Dr. Anderson will pursue his study of parasitic diseases.

E. Bruce Ashcraft has been appointed advisory engineer for the

Chemical Department of the Westinghouse Research Laboratories in Pittsburgh. Dr. Ashcraft returns to the laboratories after a four-year absence, during which he supervised research at the Oak Ridge National Laboratory, the Argonne National Laboratory, and the Westinghouse Atomic Power Division.

James A. Beal has been appointed head of the Division of Forest Insect Investigations, USDA. Dr. Beal is returning to the department from

Duke University School of Forestry, where for the past 11 years he has taught forest entomology and conducted research on forest insect problems in the Southeast. He succeeds **F. C. Craighead**, who retired last May after serving 27 years as leader of the division.

Pierre Dorolle, formerly director of health services in Indo-China, has been appointed deputy director general of the World Health Organization. Dr. Dorolle, a specialist in

tropical medicine, began serving in Indo-China in 1925. He was interned by the Japanese in 1945. After his liberation he reorganized Indo-China's medical services and arranged for their transfer to the new governments of Viet Nam, Cambodia, and Laos after World War II.

Walter N. Elsasser, of the University of Pennsylvania, has accepted a professorship in physics at the University of Utah. He will take charge of the work in theoretical physics and is planning to continue his theoretical study of the physics of the atmosphere.

Morris M. Leighton, chief of the Illinois State Geological Survey, will serve on the Advisory Committee to the U. S. Geological Survey for an additional 5-year period following 8 years' previous service.

John F. McMillan, assistant professor of biology, College of St. Thomas, St. Paul, Minnesota, will become acting chairman of the Department of Biology this month.

Ch. H. Voelker, head of the Department of Physics, Washington College, Chestertown, Maryland, has been appointed research geophysicist by The Johns Hopkins University, to work on a part-time basis. The research, to be carried on with the Chesapeake Bay Institute at Annapolis, is in the field of oceanography, and is concerned with the hydrodynamics of Chesapeake Bay and its estuaries.

Joseph S. Wade, entomologist in the U. S. Department of Agriculture, retired July 31 at the age of 70, after more than 37 years of service in the Division of Cereal and Forage Insect Investigations, Bureau of Entomology.

Fred L. Whipple, astronomer at Harvard College Observatory, has been appointed a member of the Panel on the Atmosphere, Committee on Geophysics and Geography, Research and Development Board, Department of Defense. Dr. Whipple will succeed **H. W. Wells**, scientific observer with the Department of Terrestrial Magnetism, Carnegie Institution of Washington, who has

served on the panel since its formation in December, 1948.

F. B. Wiley, chairman of the Department of Mathematics, Denison University, has retired with the title of professor emeritus and has accepted the position of chairman of the Mathematics Department of Ashland College, Ashland, Ohio.

Visitors

Man Mohan Singh, medical scientist at the Medical College at Amritsar, Punjab, India, is in the U. S., as a representative of his government, to observe American methods of teaching and research. Dr. Singh was at the University of California Medical School until September 1. He is now making a tour of medical centers including the Mayo Clinic, the Rochester Medical Center, and institutions in the New York City area.

Knut Lindblom, of the Karolinska Institute, Stockholm, will give the annual Leo G. Rigler lecture in radiology at the University of Minnesota, November 2. Dr. Lindblom will speak on "Backache." The lecture will be given in connection with a course in neuroradiology October 30-November 3 at the University Center for Continuation Study.

Recent visitors at the National Bureau of Standards were **Walter Dieminger**, director, Institut für Ionosphärenforschung, Lindau, Germany; **L. Harang**, physicist, Norwegian Defense Research Establishment, Oslo; **D. F. Martyn**, Commonwealth Scientific and Industrial Research Organization of Australia; **Peter M. Millman**, Dominion Observatory, Ontario; **Niels Venor**, civil engineer, Association of Danish Engineers, Viborg; **Nils Svartholm**, Nobel Institute for Physics, Academy of Sciences, Stockholm; **K. Weeks**, Cambridge University, England; **Hong Ki Yun**, chief, Chemistry Section, Bureau of Industry, Department of Commerce and Industry, Korea; **Bashir Ahmad** and **Nias Ahmad** from Punjab University, Pakistan; **Pierre V. Donzelot**, director of higher education, Ministry of National Education, Paris; and **L.**

G. H. Huxley, professor of physics, University of Adelaide, South Australia.

Grants and Awards

The 1950 Honor Scroll of the American Institute of Chemists, Chicago Chapter, will be presented on October 13 to Carl S. Miner, director and founder of Miner Laboratories, for his efforts in advancing the professional interests of chemists and guiding professional attitudes in younger chemists.

A special fellowship in physics has been awarded by the Illinois Institute of Technology to Ramakrishna V. Rao, physics lecturer at Andhra University, India. The award, which provides a grant of \$1,300, will enable Dr. Rao to study in the Illinois Tech Spectroscopy Laboratory with Forrester F. Cleveland, director of spectroscopy research.

Frank R. Wrenn, Jr., of the Duke University Medical School's Neurosurgery Department, has been appointed to a postdoctorate fellowship of the Atomic Energy Commission, and Byron M. Bloor, also of the Neurosurgery Department, has been appointed a Damon Runyon Clinical Research Fellow. Dr. Wrenn will attempt to develop a new method for locating brain tumors by using radioactive isotopes. The project is a collaborative effort of the Departments of Neurosurgery, Biochemistry, and Physics. Dr. Bloor will study brain tumors experimentally produced in mice to see if the type of tumor can be influenced by its location in the brain. Once produced, the tumors will be transplanted to other mice for further study.

James G. Hughes and James N. Etteldorf, associate professors of pediatrics at the University of Tennessee College of Medicine, have been awarded a research grant of \$15,179 by the National Heart Institute. The grant will be used to continue studies of high blood pressure in children, under hospital conditions, to study various diseases which produce high blood pressure in children, and for studies on the pathologic physiology of acute nephritis,

in which special attention will be given to kidney clearance, electroencephalograms (brain wave patterns), and electrocardiograms.

The John Simon Guggenheim Memorial Foundation has granted awards in mathematics to J. H. Bigelow, Institute for Advanced Study, Samuel Eilenberg, professor, Columbia University, N. E. Steenrod, associate professor, Princeton University, R. P. Boas, Jr., executive editor of *Mathematical Reviews*, Cambridge, Massachusetts, and Philip Hartman, associate professor, The Johns Hopkins University.

Colleges and Universities

The University of Kansas Medical School will present a postgraduate course in anesthesiology, September 18-20, in cooperation with the Kansas City Society of Anesthesiologists, the Kansas Medical Society, and the State Board of Health. The program has been planned to cover subjects and problems that confront part-time anesthesiologists. Information may be obtained from Dr. Paul H. Lorham, Department of Anesthesiology, University of Kansas Medical Center, Kansas City 3.

The University of Michigan's School of Natural Resources, which replaces the School of Forestry and Conservation, will offer courses in five departments this fall. The departments and their chairmen are: Forestry—Kenneth P. Davis; Wood Technology—William Kynoch; Wildlife Management—Warren W. Chase; Fisheries—Karl F. Lagler; and Conservation—Stanley A. Cain. Conservation education is being supported by a ten-year grant of \$100,000 from the Charles Lathrop Pack Forestry Foundation of New York.

Yale University, Columbia University, and the University of Uppsala, Sweden, will establish an observatory on Mount Stromlo, near Canberra, Australia. The Yale-Columbia Southern Station at Johannesburg, South Africa, will be closed, and the equipment moved to the new location, where the two American universities will install a 26-inch photographic refractor telescope. The

Australian government will construct the dome to house the instrument, and will also supply equipment, including a 74-inch reflector telescope and a Schmidt-type telescope. Plans for the cooperative arrangement were made by Dirk Brouwer, director of the Yale Observatory, Jan Schilt, director of Rutherford Observatory at Columbia, and Richard van der Riet Woolley, director of the Commonwealth Observatory. The center is expected to be ready for use by January, 1952.

Industrial Laboratories

Fisher Scientific Company, Pittsburgh, Pennsylvania, has recently developed a silicone atom model, making possible the construction of complex silicone molecules for demonstration, illustration, and research study. The new model is scaled exactly in the ratio of 100,000,000:1, so that assembled molecules can be measured by means of a meter stick.

The Kay Electric Company, Pine Brook, New Jersey, has developed an instrument that lengthens the time scale of recorded sounds by a 2:1 ratio, without altering the frequency distribution. Recorded speech seems to be spoken very slowly, although the pitch and quality of the voice remain. This instrument offers many potentialities for use in such work as general studies in phonetics, language instruction for the deaf, and foreign language instruction for those with normal hearing.

Meetings

The Executive Committee of the International Union of Pure and Applied Physics will meet in Cambridge, Mass., September 7-9. The U. S. National Committee of the IUPAP, which has recently been reconstituted, will meet with the Executive Committee on September 8. Members of the National Committee for 1950-51 are: Karl K. Darrow, chairman, Bell Telephone Laboratories; Stanley S. Ballard, Tufts College (chairman, U. S. National Committee, International Commission for Optics); Henry A. Barton, American Institute of Physics; F. G. Brickwedde, National Bureau of Standards; Robert B. Brode, Univer-

sity of California; Elmer Hutchisson, Case Institute of Technology; William Shockley, Bell Telephone Laboratories; John C. Slater, Massachusetts Institute of Technology; Merle A. Tuve, Department of Terrestrial Magnetism, Carnegie Institution; Alan T. Waterman, Office of Naval Research; and John A. Wheeler, Princeton University. Drs. Darrow and Slater are vice presidents of the IUPAP.

The Institute of Navigation, the Radio Technical Commission for Aeronautics, and the Radio Technical Commission for Marine Services will meet September 19-21, at the Hotel Astor, New York City. A series of technical symposia, followed by open discussion periods, will be held to exchange ideas on the application of electronics to related problems in air, marine, and land navigation. Paul Rosenberg, president of the Institute of Navigation, will serve as general chairman, and J. Howard Dellinger, chairman of RTCA and RTCM, has been named honorary chairman.

The Electrochemical Society will hold its 98th meeting at the Hotel Statler, Buffalo, N. Y., October 11-13. Papers on batteries, corrosion, electrodeposition, and electro-organic chemistry will be presented, and trips to various industrial plants are scheduled. The society's Acheson Medal and \$1,000 prize will be presented to George W. Vinal, of the National Bureau of Standards, on the evening of October 12. Additional information regarding the meeting may be obtained from Henry B. Linford, Secretary, Electrochemical Society, 235 West 102nd Street, New York City 25.

Deaths

Kirk Bryan, professor of geology at Harvard University since 1926, died at Cody, Wyo., on August 22. Born in Albuquerque, N. M., July 22, 1888, Dr. Bryan developed interests that were partly determined by his early environment. Best known for his interpretation of landforms, particularly those of the semiarid Southwest, he was equally expert in dating, from geological

evidence, the remains of Folsom man and his contemporaries in North America. Dr Bryan was vice president and chairman of the Section on Geology and Geography of the AAAS in 1939.

Saul Hertz, of the Harvard Medical School, died at his home in Brookline, Massachusetts, on August 2 at the age of 45. Dr. Hertz was known for his work in experimental thyroidology and was a pioneer investigator in the use of radioactive iodine.

Roy R. Kracke, dean and professor of clinical medicine at the Medical College of Alabama, died June 27 at the age of 53. Dr. Kracke was an authority in the field of hematology, and was widely known for his work with hemotherapeutic agents in the treatment of leukemia and his study of the causes of leukopenia.

The Committee on Appropriations in the House of Representatives has refused to include in the appropriations bill the half-million dollars budgeted for the **National Science Foundation**, on the grounds that even this sum of money may be used for emergency spending more effectively than for the establishment of a new agency. What the Senate will do toward restoring the proposed appropriation is unknown, but the

issue as to whether the National Science Foundation will come into existence this fiscal year or at some indeterminate date in the future will be decided about the time this news item is read in **SCIENCE**.

The American Chemical Society has established a Division of High Polymer Chemistry as the twentieth of the scientific and technical groups organized within the society. The division grew out of the success of the high polymer forums for exchange of information among chemists working in various fields, which have been held at each of the society's semiannual meetings since April, 1946. Carl Shipp Marvel, head of the Department of Organic Chemistry, University of Illinois, is chairman of the division, and Herman F. Mark, director of the Polymer Research Institute, Polytechnic Institute of Brooklyn, is secretary.

An International Index of Films and Filmstrips on Health and Welfare of Children has been prepared by the World Health Organization and Unesco. This comprehensive catalogue contains some 1,000 titles from 26 countries. It includes films made for the general public, health education shorts for children, and medical and other technical films for professional personnel. Information is included concerning content,

length, and approximate date of production of each film, as well as the address of the distribution source or the producer. The subject index includes sections on growth and development, nursery schools, child psychology, diseases and their control, education, safety, maladjusted children, welfare, nursing, medical and scientific subjects, nutrition, and public health. The publication is intended to provide health workers with a reference giving the nature and location of existing film material which may be helpful in their work. It will provide necessary preliminary information for selection and purchase of films for specific uses in various countries.

The third edition of the **International Directory of Anthropologists** has been published as a joint undertaking of the National Research Council and the American Anthropological Association. The directory, edited by Melville J. Herskovits, chairman of the Department of Anthropology, Northwestern University, contains 2,123 biographies, and includes many nationals of Far Eastern countries. The price is \$3.00. Orders should be sent to the Secretary, Division of Anthropology and Psychology, National Research Council, 2101 Constitution Avenue, Washington, D. C. This edition is limited and orders will be filled as received.



"The Rockliffe Ice Wagon," North Star plane converted by the National Research Council of Canada into a flying laboratory designed for general research on cloud physics. Flight tests will also determine the performance of experimental anti- and de-icing equipment. Most striking feature is the large "shark's fin" on top of the fuselage for use in testing the electrothermal method of wing de-icing. On either side of it are blister-type observation domes, and the propellers are fitted with blade-heater elements. J. L. Orr, head of the NRC Low Temperature Laboratory, is a member of the team of scientists that flew with the Ice Wagon to England for demonstration purposes.

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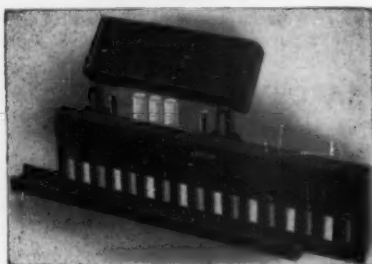
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TABLE OF CONTENTS

PART I. THE MECHANISMS OF OUR BODIES

- Ch. 1. Objectives of Biological Study
- Ch. 2. Man's Living Body
- Ch. 3. The Processes of Living
- Ch. 4. Digestion
- Ch. 5. The Chemical Machinery of the Body
- Ch. 6. Circulation

PART II. BEHAVIOR

- Ch. 7. Movement
- Ch. 8. Sensitivity
- Ch. 9. The Nervous System
- Ch. 10. Hormones
- Ch. 11. Behavior Patterns

PART III. THE INDIVIDUAL AND THE SPECIES

- Ch. 12. Reproduction
- Ch. 13. Development and Growth
- Ch. 14. Mendel and the Laws of Heredity
- Ch. 15. Gene Action and Development

PART IV. PLANT ACTIVITIES AND THE INTERDEPENDENCE OF ORGANISMS

- Ch. 16. The Basic Importance of Green Plant Cells

- Ch. 17. The Physiology of Higher Plants
- Ch. 18. The Role of Bacteria and Fungi in Nature
- Ch. 19. The Organic Community
- Ch. 20. Parasitism and Disease

PART V. THE EVOLUTION OF PLANTS

- Ch. 21. Evolutionary Change
- Ch. 22. The Thallophytes
- Ch. 23. Vegetative Evolution of Higher Plants
- Ch. 24. Evolution of Reproduction in Higher Plants

PART VI. THE EVOLUTION OF ANIMALS

- Ch. 25. First Steps in Animal Evolution
- Ch. 26. Evolution of the Arthropods
- Ch. 27. Sidelines in Invertebrate Evolution
- Ch. 28. Vertebrate Ancestry
- Ch. 29. The Evolution of Mammals and Man
- Ch. 30. The Mechanisms and Control of Evolution

APPENDIX: BASIC CONCEPTS FROM CHEMISTRY AND PHYSICS

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GLOSSARY-INDEX

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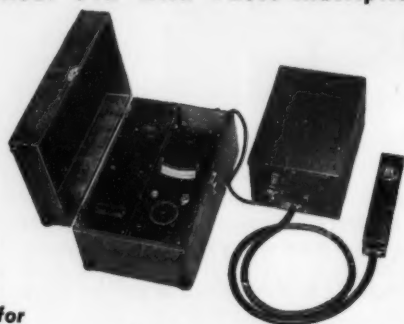
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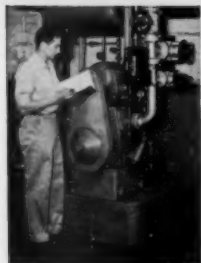
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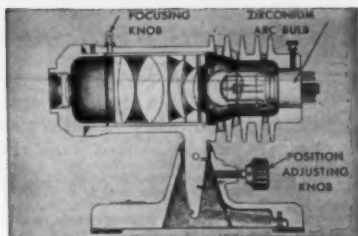
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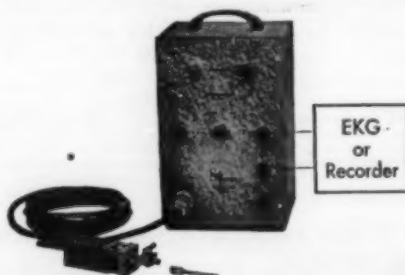
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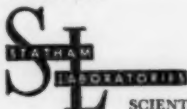


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1. Rate: 15¢ per word, minimum charge \$3.00 for each insertion. If desired, a "Box Number" will be supplied, so that replies can be directed to SCIENCE for immediate forwarding. Such service counts as 8 words (e.g., a 25-word ad, plus a "Box Number", equals 33 words). All ads will be set in regular, uniform type, without display; the first word, only, in bold face type.

For display ads, using type larger or of a different style than the uniform settings, enclosed with separate border rules, the rate is \$16.00 per inch; no extra charge for "Box Numbers".

2. Advance Payment: All Personnel Placement ads, classified or display, must be accompanied by correct remittance, made payable to SCIENCE. Insertion can not be made until payment is received.

3. Closing Date: Advertisements must be received by SCIENCE, 1515 Mass. Ave., N.W., Washington 5, D. C., together with advance remittance, positively not later than 14 days preceding date of publication (Friday of every week).

POSITIONS WANTED

Bacteriologist: Ph.D. Yale, 1947. Age 31. Desires academic position. Teaching and research experience in bacteriology and mycology. Publications on the nature of virulence of microorganisms. Box 287, SCIENCE. X

Biochemist, Ph.D., desires research and/or teaching position; experienced in nucleic acid and enzyme chemistry; publications. Box 289, SCIENCE. X

Botanist, Ph.D., Ten years experience, General Botany, Mycology, Taxonomy, etc. Available Fall term. Box 288, SCIENCE. X

Chemist, B.S.; organic synthesis; tracer techniques with radioisotopes; interested in Medical Research; Box 290, SCIENCE. X

Ecologist; age 30, with A.B. in Geology, Ph.D. in Botany, graduate work in wildlife management, and interest in anthropology, wants to do teaching or research in ecology. Has experience as consultant with government commission and as resident ecologist on grass farm for 3 years. Have publications, best training and references. Box 291, SCIENCE. X

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The Market Place

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1. Rate: 20¢ per word for classified ads, minimum charge \$5.00 for each insertion. Such ads are set in uniform style, without display; the first word, only, in bold face type.

For display ads, using type larger or of a different style than the uniform classified settings, and entirely enclosed with separate rules, rates are as follows:

Single insertion	\$16.00 per inch
7 times in 1 year	14.50 per inch
13 times in 1 year	13.00 per inch
26 times in 1 year	11.50 per inch
52 times in 1 year	10.00 per inch

2. Payment: For all classified ads, payment in advance is required, before insertion can be made. Such advance remittances should be made payable to SCIENCE, and forwarded with advertising "copy" instructions. For display advertisers, monthly invoices will be sent on a charge account basis—providing satisfactory credit is established.

3. Closing Date: Classified advertisements must be received by SCIENCE, 1515 Massachusetts Avenue, N.W., Washington 5, D. C., together with advance remittance, positively not later than 14 days preceding date of publication (Friday of every week).

For proof service on display ads complete "copy" instructions must reach the publication offices of SCIENCE, 1515 Massachusetts Avenue, N.W., Washington 5, D. C., not later than 4 weeks preceding date of publication.

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Your sets and files of scientific journals

are needed by our library and institutional customers. Please send us lists and description of periodical files you are willing to sell at high market prices. J. S. CANNER AND COMPANY, 909 Boylston Street, Boston 15, Massachusetts. tf

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The Market Place

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4. Advance Registrants will have the same privileges of receiving a map and directory of points of interest of Cleveland, literature, radio broadcast tickets, etc. At the convenience of Advance Registrants, these will be distributed from the Main Registration in the Public Auditorium—the location of the Annual Science Exposition, the Visible Directory, and the Science Theatre. Admission to the splendid series of latest scientific films will be free to all Registrants.

At the 1949 Meeting, the Council of the AAAS voted overwhelmingly to continue Advance Registration. To insure its prompt receipt, the General Program will be sent by *first class mail* December 1-4, 1950—which is also the *closing date* for Advance Registration.

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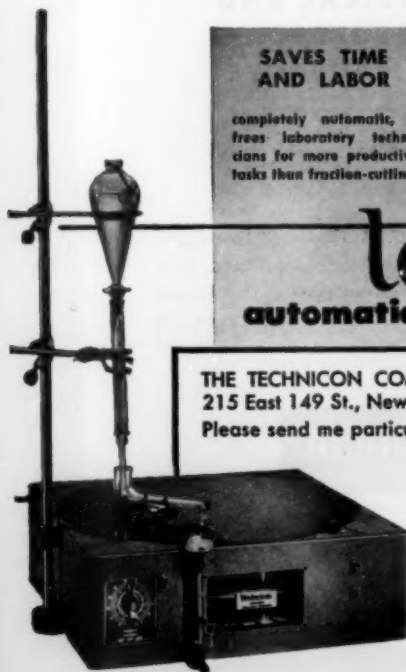
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